THE ROLE OF SOIL ORGANIC MATTER IN THE SUPPLY OF SULPHUR TO PLANTS

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ABSTRACT

Sulphur balance studies in S.E. Scotland and W. Scotland showed no ryegrass yield response to added sulphur. However added sulphur improved the nutritional quality of the herbage at all sites. The sulphur sufficiency of the S.E. Scotland sites was due to significant contributions of soil sulphur (approximately 17 kgS/ha/growing season in Berwickshire and 8 kgS/ha/growing season in Midlothian). Added sulphur did not increase concentrations of Kjeldahl nitrogen or have any effect on nitrate concentrations in herbage. Sulphur, applied in the spring, was lost from the topsoil by the following spring. Although no yield responses were obtained in the field, the same S.E. Scotland soils exhibited ryegrass yield responses to added sulphur in pot experiments (up to 36 percent yield increases were obtained). Other Scottish soils also showed marked ryegrass yield increases to added sulphur. Pot experiments showed that the best indicators of plant sulphur status were the extractable plant sulphate expressed as a percentage of the total sulphur and the plant extractable sulphate. Critical levels were 30 percent and 600 µgS/gD.M. respectively. Total plant sulphur proved to be a poor indication of plant sulphur status. Only when plant extractable sulphate fell to below 50 µgS/gD.M. was there a significant increase in herbage nitrate levels.

Incubation studies showed that air dried soils exhibited an initial flush of mineralised sulphur. Added glucose-carbon consistently reduced the amounts of sulphur mineralised and even caused immobilisation in the Whitsome soil. Five soils showed increased net sulphur mineralisation with increase of temperature in the range $5^{\circ}C$-$30^{\circ}C$. Total soil sulphur and extractable soil sulphur correlated with net sulphur mineralisation (as measured by incubation). Soil carbon: sulphur ratios did not correlate with sulphur mineralisation. Sulphur-35 experiments showed that net sulphur mineralisation was unrelated to gross sulphur mineralisation (whilst added glucose-carbon increased sulphur turnover, net mineralisation decreased). The majority of the incorporated sulphur-35 was recovered from the HI-reducible sulphur, which showed the more transformational activity. The recently incorporated sulphur was shown to be more labile than the indigenous soil sulphur. This recently incorporated sulphur was very susceptable to breakdown to sulphate on air-drying of the soil. A gel permeation technique was developed capable of fractionating extracted soil organic sulphur on a basis of molecular weight. Organic sulphur compounds $>$ 200,000 molecular weight consistently contained a large proportion of HI-reducible sulphur. Recently incorporated sulphur-35 was found in organic molecules of all molecular weights within the range $10,000$-10^{6}.

DECLARATION

I hereby declare that this thesis was composed by myself and the work described was carried out entirely by myself.

J.I. KEER

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CONTENTS

1. INTRODUCTION

Sulphur has long been known (since the time of Liebig) to be an essential element required by plants and animals for normal growth. Indeed sulphur is part of the cysteine and methionine molecules, both of which are amino acids essential in the make up of proteins. Earlier work in the field of soil sulphur studies may have been prompted solely by academic interests, but over the last twenty years increasing numbers of sulphur deficient areas have been reported and this has necessitated a greater understanding of the soil sulphur cycle. Sulphur deficient areas can occur in all parts of the world, for example; Spain, France and Germany in Europe, Norway and Sweden in Scandinavia, many areas of Africa, south-eastern U.S.A., large areas in New Zealand and Australia and Canada (Coleman, 1966). Many other areas of sulphur deficiency or marginal sufficiency undoubtedly exist but, as yet, have not been identified. Whilst it was previously supposed that Britain, with its dense industrialisation would be immune to sulphur deficiency it has been shown recently (Cowling and Jones, 1970; Jones et al., 1972; McLaren, 1975; Williams, 1975; Scott, 1979 and Scott and Munro, 1979) that some areas of Britain have at best, only marginal sulphur sufficiency. Also herbage containing insufficient sulphur, could adversely affect animal nutrition (McLaren, 1976).

Such marginal sufficiencies were demonstrated by experiments carried out in filtered air chambers (thus eliminating atmospheric inputs of sulphur) where yield responses to added sulphur were obtained for most soils of widespread location in England and Wales (Cowling and Jones, 1970). These responses shown by perennial ryegrass to added sulphur were particularly significant when high nitrogen dressings were applied. Therefore the sulphur derived from the soil reservoir alone, is inadequate for maximum yields of high yielding perennial ryegrass (extrapolation to cover other crops would be reasonable). Jones et al., (1972) found geographical trends in the soil sulphur status of British soils with the calcareous, dry, soils of eastern Britain containing less organic sulphur than their western counterparts. Also because they also contained less free iron oxides and had higher pH values, less adsorbed sulphate was detected. This is in direct comparison with the soils of the western areas which contained more organic and adsorbed sulphur. From these observations, Jones et al., (1972) concluded that fertiliser and atmospheric sulphur additions were of widespread importance, particularly with regard to the soils of eastern England. Indeed many areas of the U.K. are probably almost totally reliant on atmospheric sulphur additions to ensure that sufficient sulphur is made available to the growing plant. A sulphur survey of south-eastern Scotland (McLaren, 1975), revealed that many soils contained less than 12 $\mu g/g$ of extractable sulphur - a

level which indicated that yield responses to added sulphur might be obtained. Also plant sulphur contents were low, suggesting that only marginal levels of sulphur were available. Scott (1979) also worked with Scottish soils and found oat yield responses to added sulphur where rainfall sulphur was excluded from the pots. A survey of north-west Pembrokeshire showed that 14% of the herbage sampled was sulphur deficient (Williams, 1975), and a positive correlation between herbage sulphur content, and soil organic matter content was obtained, indicating that soil organic matter might be a significant source of available sulphur. The importance of this source is greater where atmospheric and fertiliser inputs are low. The results of pot trials and field surveys indicate that the statement, "where rain supplies 12 kgS/ha/yr sulphur deficiency is unlikely" (Cooke 1975) has become out-dated.

Although the significance of the inputs of sulphur from rain, dry deposition and air have been reported (Jones et al., 1972 and Scott, 1979) there is remarkably little data concerning amounts of sulphur supplied to the soil and plant from these sources. Stevenson (1968) has collected data from 1959-1964 which gave concentrations of sulphur in rain-water and air. The lowest concentrations (1.4 — 2.0 $\mu g\ S/m^3$) found were in central Ireland, and central southern Scotland, indicating that inland non-industrial areas can be expected to receive low atmospheric inputs. It has been calculated that central Ireland receives 7-9 kgS/ha/yr, whereas coastal regions receive

20-28 kg/S/ha/yr (Hanley and Tierney, 1969) from rainfall. However, only about 30 percent of this sulphur is precipitated during the growing season. Some monitoring of rainfall sulphur concentrations has been carried out abroad (Seim et al., 1969; Walker, 1969), the results varying greatly with location, especially with regard to proximity to industrial sites. A rural site in Minnesota received 5.4 kgS/ha/yr in comparison to a metropolitan site which received upwards of 30 kgS/ha/yr (Seim et al., 1969). Similarly rain and snow sulphur concentrations in central Alberta (non-industrialised) rarely exceeded 1.5 μgS/g which is the equivalent to 2.2 — 4.4 kgS/ha/yr compared with the range found in Sweden of 1.9 — 14.7 kgS/ha/yr. Concentrations of sulphur in the air have been often measured but usually in large urban areas, where the primary aim was pollution control, rather than measurement of additions of agriculturally useful sulphur. Stevenson (1968) has, however, collected data from diverse locations (both rural and urban areas) and found that an annual trend was always present with lower concentrations during the summer and higher levels in winter. This was probably due to increased use of sulphur containing fuels during winter. Again concentrations varied with location and proximity to industry. The lowest aerial concentrations were found in central southern Scotland (5 $\rightarrow$ 10 μgS/m^3) and Ireland (3-17 μgS/m^3). The highest aerial sulphur concentrations were found at Leeds and Rothamsted (50-60 μgS/m^3).

The difficulty in converting aerial sulphur concentrations into amounts of sulphur received by the soil/plant system has been stressed by meteorologists (Bromfield and Williams 1974; Fowler and Unsworth, 1974). For one given aerial sulphur concentration the amount of sulphur being deposited depends on the deposition velocity which is a function of the nature of the surface of the sink. For example Lockyer et al., (1978) noted that soil type had no effect upon deposition velocity although higher soil pH values increased deposition velocity. Fowler and Unsworth (1974) suggested that wet surfaces enhanced dry deposition rates and Bromfield and Williams (1974) indicated that deposition rates were two or three times higher onto a crop surface as opposed to a bare soil surface. Fowler and Unsworth (1974) calculated that Britain receives 24 kgS/ha/yr assuming a deposition velocity of 3.6 mm s^{-1} and that the sink surface is a uniform arable crop. However, such a value is of limited use since the aerial concentration of sulphur varies greatly with location and hence inputs of aerial sulphur vary accordingly. Dry deposition of sulphur has also been measured directly by measuring the total sulphur content of 2 g of finely ground soil before and after a three month exposure to the air (the experiment was conducted inside a Stevenson screen). Values obtained were the equivalent to 18.1 kgS/ha/yr at Saxmundham and 27.5 kgS/ha/yr at Rothamsted. These directly measured values agree reasonably well with the values obtained indirectly using

aerial concentrations of sulphur and deposition velocities. Clearly more extensive and localised data is needed for Britain before the dry deposition of sulphur for a given region can be confidently forecasted.

Although the possibilities of sulphur deficiency in many areas of the world are only now being realised and are often only marginal, current agricultural, industrial and social trends are very likely to worsen the situation. Thus the increasing frequency of sulphur deficiency reports stem not only from increasing awareness of the problem but also from changing fertiliser practices and the desire for a pollution-free environment. At one time ammonium sulphate was a widely used nitrogen fertiliser but because of associated soil acidity problems its use is currently much less extensive. Also ammonium sulphate has been replaced as a source of nitrogen by ammonium nitrate which gives a higher nitrogen analysis and therefore cuts down on the bulk needed, and so reduces shipping costs. The super-phosphate, originally manufactured by John Lawes in 1840 contained much sulphate as a result of the use of sulphuric acid in its manufacture. These sulphate-containing phosphatic fertilisers have largely been replaced by ammonium phosphates and triple super-phosphates which contain very little sulphur. Another potential input into the pool of soil sulphur which has been recently reduced, arose from the replacement of sulphur used as a fungicide with synthetic organic compounds. The agronomic advances made in recent years have greatly increased crop

yields therefore more sulphur is removed from the soil in the useful yield. This greater output of sulphur coupled with higher applications of sulphur free, high analysis fertilisers will widen the C:N:S: ratios of the soil and plant. Thus the nutritive value of the plant could be decreased and a high C:N:S: ratio in the soil could effectively reduce supplies of sulphate made available by mineralisation. Another possible contributory factor to the increase in reports of sulphur deficiency is the replacement of relatively high sulphur content fuels such as wood and coal with natural gas and atomic energy (Coleman, 1966). Coleman (1966) also noted that domestic use of coal is rapidly diminishing which reduces the even spread of atmospheric sulphur. Thus sulphur distribution is such that some areas will receive large excesses, whilst other areas are likely to become deficient. All the above factors contribute to more frequent reports of sulphur deficiency making an understanding of the forms of soil sulphur and the relative contributions of atmospheric and soil sulphur to plant nutrition of considerable importance.

The literature review has been written with particular reference to the arable soils of humid regions. This is because it is on these soils that deficiencies are prevalent and also where much of the world's food is grown. As a consequence, consideration of the microbial reduction processes involving sulphur have been omitted as their significance in arable soils is very limited (Swaby

and Fedel, 1973). The review attempts to stress the cyclical nature of sulphur in the soil/plant/atmosphere system and highlights the problems of research into organic soil sulphur which is the key to finding a reliable method to evaluate the sulphur status of soils.

2. LITERATURE REVIEW

2.1 Analytical Techniques used for the Determination of Sulphur

The diagnoses of sulphur deficiency, especially with regard to borderline cases, necessarily involves the estimation of sulphur. This may be the analysis of plant tissues, of soil extracts, or the atmospheric inputs such as rain-water, air and dry deposition. It was clear that a very accurate and sensitive method of sulphur determination was needed before any predictions regarding the sulphur status of soils could be made. This is because soils of the humid regions frequently contain $0 \rightarrow 15$ µg/g of sulphate-S extractable by the more commonly employed reagents, and the difference between a sulphur sufficient soil and one which is marginally deficient may amount to a difference of only 3 µg/g of extractable sulphate-S. Unfortunately, research into soil sulphur has been severely hindered by the lack of a rapid and reliable analytical technique (Sinclair, 1973). Some of the earliest techniques were far from adequate as they involved a gravimetric procedure in which the sulphur (as sulphate) was precipitated as barium sulphate. Since then, however, most new techniques have relied upon the extremely low solubility product of barium sulphate in aqueous solutions. Methods available include measurement of stable barium sulphate suspensions by nephelometry or turbidimetry, measurement of uncombined barium ions after

precipitation of barium sulphate, using various colorimetric methods or atomic absorption spectrophotemetry. The only real alternative to the methods utilising the insolubility of barium sulphate involve a lengthy reduction to hydrogen sulphide. The sulphide can then be very accurately estimated by colorimetric or volumetric methods. The advantages and disadvantages of the above methods will be expanded upon in this chapter with special reference to the analysis of soil and plant material.

2.1.1 Determination of sulphur by manual turbidimetric methods

The turbidimetric method originated in the anals of medicine and was first used in connection with soils by Chesin and Yien in 1950. These workers recognised the insensitivity of the gravimetric method for the assessment of soil sulphur status and being the only proven method available, it clearly had to be modified and improved. This they did by extracting soils with Morgans solution, adding barium chloride crystals of a known particle size range, stabilising the suspension with gum arabic (a protective colloid) and measuring the turbidity with a photo-electric colorimeter. The method compared well with gravimetric results although they were always consistently lower (probably due to co-precipitation of extracted materials with barium sulphate in the gravimetric method). The authors claimed good replication and accuracy combined with sensitivity. Later, Hesse (1957) showed that the method was unreliable

and gave erroneous results, due to the colloidal organic matter which is extracted by Morgans solution. Low results would be obtained in the 0-10 µg/g sulphate-S range since the organic matter acted as a protective colloid. High results could be expected when high sulphate levels were incurred due to co-precipitation of the colloidal organic matter with the barium sulphate particles. Hesse (1957) therefore began looking for ways of removing the organic matter instead of seeking a more suitable extractant which might have been more pertinent. However, co-precipitation of organic matter with ferric hydroxide and subsequent removal by filtration gave increased sensitivity and more reliability than the original method proposed by Chesin and Yien (1950). Hesse (1957) additionally found that activated charcoal removed insufficient organic materials and that removal by oxidation with hydrogen peroxide liberated organic sulphur as sulphates. In 1959 (Butters and Chenery) the turbidimetric method was made more versatile so that total sulphur in soils and plants could be measured. The soil and plant samples were oxidised by heating to 500°C with pure magnesium nitrate and digested with nitric acid. Such a procedure converted organic sulphur to sulphate-sulphur which was readily determined by the turbidimetric method. The same group of workers also investigated factors which affected the reproducibility of the somewhat unreliable method of turbidimetry. It was found (Butters and Chenery, 1959) that the size of barium chloride

crystals used, standing time of the suspension and the presence of the interfering cations, Fe^{3+} and Mn^{3+}, all affected the optical properties of the suspension. Thus it became clear that if independently obtained results could be usefully compared, meticulous standardisation of techniques must be ensured. Another fault with the method at that time was that Morgans extract, being of a low pH, greatly overestimated amounts of available soil sulphur (see chapter on assessment of soil sulphur status). Therefore until the turbidimetric method could be made more sensitive, milder extractants could not be used. The necessary refinement was made by Massoumi and Cornfield (1963) and good sensitivity over the 2-10 µg/ml SO_4^{2-}-S was obtained. This increased sensitivity was achieved by the incorporation of a very dilute seed suspension of barium sulphate. These workers also found that purified animal charcoal was acceptable for decolourising and removing soluble and colloidal organic molecules which interfered with the determination. Even with these refinements, most workers employed the reduction method of Johnson and Nishita, (1952) as erratic results were often obtained from turbidimetry (Garrido, 1964).

2.1.2 Determination of sulphur by automated turbidimetric methods

A complete examination of the turbidimetric technique was made (Garrido, 1964) and a new protective colloid, Tween 80 (a surface active substance) was used with

success. The greatest advance in the development of the turbidimetric method was probably the advent of automated analysis. The intention was to make the analysis more rapid and convenient, but because exact reaction conditions could be easily duplicated the erratic nature of the method was largely overcome. Mottershead (1971), was the first to automate the technique but he did not critically test the method at the low concentration levels involved in soil sulphur status assessment. However, it was suggested that continuous pumping of additional sulphate-sulphur would ensure that the barium sulphate solubility product was exceeded where low sample sulphate levels were found. Several workers (Mottershead, 1971; Basson and Bö hmer, 1972) incurred problems associated with the adherence of barium sulphate to solid surfaces (Gibson, Bailey, Furkert and Giltrap, 1974). This caused a drifting of the baseline over relatively short periods of operation due to coating of the photometer cell. This coating was periodically removed by solution in alkaline E.D.T.A. Some workers employed a nephelometer in preference to a colorimeter to measure the turbidity of the barium sulphate suspension (Bettany and Halstead, 1972; Basson and Bö hmer, 1972). This technique measures the amount of light scattering rather than the amount of light transmittance measured by a colorimeter. Bettany and Halstead (1972) claimed that their automatic nephelometry method measured 0.25 µg/ml sulphur with good recoveries of standard additions of 0.25, 0.5 and 0.75 µg/ml

sulphur. In this method polyvinyl alcohol was used to stabilise the suspension after organic matter was removed by sodium peroxide treatment.

More recent work (Basson and Boehmer, 1972; Sinclair, 1973) has mostly cured the earlier problems of coating, sample carry over and background noise. Flow rates of reagents were increased to ensure completely homogenous suspensions and to eliminate coating while longer wash times with a double probe system prevented sample carry over. Recently, (Ogner and Haugen, 1977) a simple dialyser has been incorporated into the automated system to eliminate the need for prior sample treatments aimed at removing extracted organic matter. However, whilst an efficient removal of carbon-containing compounds was achieved, some loss of sulphate excluded its use in the 0-10 $\mu g/ml$ SO_4^{2-}-S range. In conclusion, the workers reported that the method was more reliable than the turbidimetric method after a colour correction. This observation did not encourage the use of the dialyser since a colour correction cannot account for the interference caused by colloidal organic matter (Hesse, 1957; Massoumi and Cornfield, 1963). Recently, continuous flow injection turbidimetry has been developed which allows the very fast rate of analysis of 180 samples per hour (Krug et al., 1977).

2.1.3 Determination of sulphur by reduction to hydrogen sulphide.

While the turbidimetric method was still in its infancy the erratic results obtained rarely encouraged

confidence and not until Johnson and Nishita (1952) published their reduction method, was there a suitable alternative. The method measured 0.5-300 µgS/g with good precision. Unlike the turbidimetric method, which measures only SO_4^{2-}-S the reduction technique includes all sulphur compounds which are reduced to hydrogen sulphide by a mixture of hydriodic acid, formic acid and red phosphor us. It was probably due to the accuracy of this method that HI-reducible sulphur was so unanimously accepted as a soil organic sulphur fraction. The hydrogen sulphide evolved on reduction was adsorbed and measured colorimetrically after complexing with methylene blue. The method gives a final value which includes sulphate, sulphide, sulphite, thiosulphate and most organic compounds where the sulphur is not directly bonded to the carbon atom (Bird and Fountain, 1970). Johnson and Nishita (1952) showed that added cystine, cysteine, taurine and methionine were stable to the reduction procedure. The exposition of such an accurate and sensitive method was a big advancement in predicting potentially sulphur deficient areas. The method also had the advantage of not suffering from interfering ions, but length of determination was the biggest problem which is why workers have recently favoured the automated turbidimetric method. The reduction method is versatile and can be used for plant analysis when the determination of total sulphur is required (preliminary ashing, wet ashing or chemical oxidation is needed). The need to prepare plant material so that all the sulphur present is

reducible has caused some problems (Steinbergs et al.,(1962) Bird and Fountain, 1970). Since ashing could result in the loss of some of the more volatile sulphur compounds, the method of Steinbergs et al., (1962) was most frequently employed. This method can be used to obtain values of total sulphur for both soils and plants, and entailed an oxidation at 550°C with sodium hydrogen carbonate and silver oxide.

The reduction method proposed by Johnson and Nishita (1952) was still being used although some modifications, aimed at reducing the time needed to perform the reduction, have been made. For instance Archer (1956) introduced a titrimetric finish after adsorption of hydrogen sulphide in N sodium hydroxide. This reagent showed great affinity for hydrogen sulphide, and the gas could be flushed out of the reaction vessel much faster without fear of loss arising from incomplete absorption. The sulphide solution is titrated with Hg (II) ions until all the sulphide is precipitated and a red dithionate formation indicates complete precipitation. This modification reduced the sensitivity of the method so that only samples above 20 µgS/g could be measured accurately. Another colorimetric finish involved the formation of bismuth sulphide, a colloid stabilised with gelatin, but again although rapidity is increased a six-fold loss in sensitivity was incurred (Dean, 1966).

2.1.4 Determination of sulphur using Barium-133.

A new method offering great potential sensitivity has been published by Kao et al., (1971) in which sulphate was measured as the $^{133}BaSO_4$ precipitate. The method was specifically designed for the 0-10 µgS/g range where existing techniques were often inadequate. Barium-133 is a useful radio-isotope as it has a long half-life and energetic gamma radiation emission. The soil extractant employed was 5mM $CaCl_2$. After passing the extractant through a cation exchanger an aliquot was placed on an aluminium dish and dried following the addition of ^{133}barium chloride. Since the ^{133}barium sulphate adhered strongly to the dish, washing to remove excess ^{133}barium chloride was a simple process. A liquid scintillation counter enabled the detection of 1 µgS/g and sulphur standards gave a straight line relationship between amount of sulphur and count rate. This method appeared to offer the research worker a useful new technique with much potential and versatility.

2.1.5 Determination of sulphur by methods involving the estimation of uncombined Ba^{2+} ions.

Other automated methods for sulphate determination are available (Persson, 1966; McSwain and Watrous, 1974) which rely on the formation of a coloured complex with barium ions. A known concentration of a soluble barium salt is added to the sample and the barium not used up in the barium sulphate precipitation is determined colorimetrically.

Persson (1966) recommended the use of Thorin, 1-(o-arsonophenylazo)-2-napthol-3, 6 disulphonic acid where an orange colour is formed in an organic medium of isopropanol. Another alternative is the use of methylthymol blue (McSwain and Watrous, 1974) which is sufficiently sensitive for use in the 0-10 µgS/g range. Whilst both methods readily lend themselves to automated analysis their versatility was very restricted. Most cations interfered, as did phosphate and chloride, making painstaking sample preparation necessary when plant and soil samples were being analysed. However, the excellent sensitivity of this group of methods enabled "relatively clean" samples, such as fresh water and rain-water, to be very accurately analysed.

2.1.6 Determination of sulphur by X-ray fluorescence spectrometry (X.R.F.)

X-ray fluorescence spectrometry has recently been developed for the estimation of light elements in plant material. The method has the advantages of being non-destructive, potentially very sensitive and versatile as well as being almost entirely free from interferences. Since the method is specific for sulphur atoms the result obtained is necessarily a total sulphur value. Roberts and Koehler (1968) made use of XRF for measuring sulphur in soil extracts. After an extraction with 5mM magnesium chloride and a matrix adjustment to decrease X-ray adsorption the soil extract was evaporated onto a Mylar film and irradiated. Counts were found to be directly

proportional to the sulphur present and straight line calibration curves were obtained. Good accuracy and sensitivity with soil samples containing between 0.5 and 14 µgS/g was reported. Gibson et al., (1974) concentrated the sulphate present in a monocalcium phosphate extract by passing the extract through an anion-exchange paper and subsequently irradiating the paper. However, the sulphate ions did not completely penetrate the exchange paper and therefore a non-uniform distribution of sulphur might be presented to the X-rays. Indeed the workers found only weak agreement with chemical methods and did not test the method below 10 µgS/g. Thus it can be seen that the greatest problem of XRF techniques concerns sample preparation. This preparation was simplified where solids were concerned as the method of Evans (1970) illustrated. An equal quantity of plant material and cellulose was finely ground, reducing particle size effects, and compressed into a homogenous pellet suitable for irradiation. Many plant species were analysed and in each case no correction for matrix or inter-element effects were needed. Bergseth and Kristiansen (1978) compared the X-ray fluorescence method for determining sulphur in soils with an indirect atomic adsorption method and a nephelometric method. Satisfactory agreement between the X-ray and chemical methods was obtained when the soil pellets were prepared in wax (necessary to prevent uptake of gaseous sulphur from the vacuum oil). XRF appears to be a most valuable

tool for the estimation of total sulphur in soil (solid samples) and plant material but until a quick, efficient sample preparation method is developed for use with soil extracts, chemical methods probably remain preferable.

Several other methods of sulphate analysis are available as reviewed by Little (1953) such as precipitation of benzidine sulphate and the use of internal indicators such as rhodizonic acid. Stephen (1970) developed a reagent extremely sensitive to sulphate, claiming 0.05 $\mu g SO_4^{2-}$-S/g could be measured, however, the numerous interfering ions preclude its use with plant and soil samples.

To summarise; useful analytical techniques for sulphur determination in soils and plants are limited to turbidimetric, HI-reduction and XRF methods. Automated turbidimetry offers versatility, sensitivity over low sulphur concentrations, little interference and rapidity and therefore is perhaps the most useful method overall. Hydriodic acid-reduction has the one major disadvantage of being slow and also measures a somewhat ill-defined class of sulphur compounds (unless a thorough pre-treatment is performed). X-ray fluorescence is a quick accurate method when applied to solid samples and is useful in that total sulphur is measured (all other methods entail lengthy sample pre-treatments before a total value can be obtained).

2.2 The Nature of Soil Organic Sulphur

It is generally known that most soil sulphur occurs in organic combination but knowledge of these compounds is very limited. This lack of knowledge originates from a failure to develop an extraction procedure which liberates sulphur compounds from other soil constituents in an unaltered form. It is also one reason why the general advancement of knowledge of soil organic matter has been hindered. The extent of this problem was fully realised by Whitehead (1960), who set out to identify the forms of organic sulphur in soils, but instead wrote a thesis on methods of soil sulphur extraction, none of which proved to be entirely successful! However, this work did make several useful points regarding soil organic sulphur. Firstly, Whitehead (1960) eliminated the lipid fraction (extractable in ether) as a potential source of sulphur as less than 0.1 percent of the total organic sulphur was detected here. Secondly, the amounts of sulphur occurring as the amino acids methionine and cystine/cysteine were much smaller than previously supposed. To explain this observation it was proposed that the sulphur containing amino-acids rapidly underwent condensation reactions with quinones (from decomposition of lignins) thus becoming incorporated into the humus with subsequent stability to decomposition (Whitehead 1960 and 1964). This theory, to the present moment, remains unsubstantiated by direct evidence, but no evidence for its rejection has been obtained. The only indirect

evidence comes from an early study (Shorey, 1913) when trithiobenzaldehyde was isolated from soil, suggesting a similar condensation reaction between hydrogen sulphide and the aldehyde originating from lignin. Another pointer in favour of this concept was the finding of 25 percent of the organic sulphur occurring as sulphur directly bonded to carbon in Canadian surface soils (De Long and Lowe, 1962) as determined by the Raney nickel desulphurisation procedure.

2.2.1 The occurrence of soil sulphur combined as amino-acids.

When considering amino acid sources of soil organic sulphur the literature is commonly hazy and often fails to differentiate between free, uncombined amino-acids and amino-acids which were the result of laboratory hydrolysis. However, since amino-acids are readily utilised by micro-organisms one would not expect large quantities of free amino-acids to occur in the soil. Work by Putnam and Schmidt (1959) has supported such expectations. Other work has been performed to examine the chemical forms and amounts of amino-acids occurring in soil hydrolysates. Freney *et al.*, (1972) used ion exchange chromatography and X-ray fluorescence to detect sulphur containing amino-acids. These methods were satisfactory but needed development; findings indicated that 26 percent of the total soil sulphur occurred as the amino-acids (bonded together as proteins and peptides) methionine and cystine. This corresponded to approximately half of the total

carbon-bonded sulphur and was much larger than estimates based on N:S ratios (Whitehead, 1964) and amino acid analysis. Since reagents capable of hydrolysis were employed, usually 6N HCl, experimental artefacts cannot be ignored, and amounts of amino-acid sulphur could be over-estimated.

2.2.2 The fractionation of soil organic sulphur into HI-reducible sulphur and carbon-bonded sulphur

Over the last fifteen years a widely used fractionation procedure for soil organic sulphur has been evolved. This procedure is rather empirical in nature such that similar and meaningful classes of compounds are rarely found occurring in one fraction, and are often unrelated to soil properties. Most workers fractionate organic sulphur into that which is reduced by hydriodic acid and that which is desulphurised by reduction with Raney nickel (first reported as a direct method for the determination of carbon-bonded sulphur by De Long and Lowe, 1962). However, the summation of the two fractions mentioned above rarely accounts for more than 90 percent of the total organic sulphur (Lowe, 1965 and Freney *et al.*, 1970) which led Lowe (1965) to suggest the presence of a very inert sulphur fraction or a preparation artefact. Another possible explanation is that the Raney nickel does not recover all the sulphur which is directly bonded to carbon. Indeed, Freney *et al.*, (1970) found that aliphatic sulphones and aliphatic sulphonic acids (both carbon-bonded sulphur compounds) both remain unrecovered

by the nickel reduction. This lack of specificity also extended to inorganic forms of sulphur, with elemental sulphur, metabisulphite, thiosulphate and sulphate all being included in the Raney nickel reducible fraction. However, this is not a serious limitation as these inorganic forms of sulphur are rarely significant in soils unfertilised with elemental sulphur. Freney et al., (1970) found for all soils that the calculated carbon-bonded sulphur fraction (total organic sulphur minus HI-reducible sulphur) always exceeded the directly measured quantity and attempted to reduce this disparity. However, even when iron and manganese interferences were overcome by increasing the reducing power of the catalyst (by adding Na/Al alloy plus sodium hydroxide) only a 30 percent recovery of the calculated carbon-bonded sulphur was obtained suggesting the presence of a third distinct sulphur fraction. However this work incorporated the use of sodium hydroxide which is a powerful hydrolysing agent capable of drastically altering naturally occurring soil organic sulphur.

Freney (1957 and 1961) was the first worker to suggest the use of hydriodic acid to fractionate soil organic sulphur as he believed organically bound sulphates formed a considerable portion of the soil organic sulphur. The use of this procedure was quickly adopted and is still extensively used even though no individual sulphur compounds in this fraction have been identified. It was found that 50 percent of the organic sulphur was HI-

reducible in some Australian soils. A similar proportion of HI-reducible sulphur was found in Canadian soils (De Long and Lowe, 1962, Lowe 1964). The analytical method (Johnson and Nishita, 1952) involves reduction of sulphur to hydrogen sulphide which is then determined titrimetrically using methylene blue. However the reduction includes inorganic sulphur which necessitates a separate determination of inorganic sulphur. When the amount of inorganic sulphate was deducted from the HI-reducible sulphur, strong correlation was obtained between this residual quantity and both total carbon and total nitrogen indicating that the HI-reducible sulphur is organically combined (Freney, 1961). In the same report (Freney, 1961) it was shown that the HI-reducible sulphur mostly occurred in the fulvic acid fraction.

Sulphated polysaccharides have often been put forward as significant forms of soil organic sulphur (Freney et al., 1962; Whitehead, 1964) but later work (Lowe, 1965) with Alberta soils indicated that less than 2 percent of the total sulphur occurred in such forms (as extractable in hot water). The largest amounts of these sulphated polysaccharides were found in organic horizons, a trend which is followed by all soil polysaccharides.

2.2.3 Procedures for extracting soil organic sulphur

The arbitrariness of the fractionation procedures outlined above has resulted in efforts being made to find a more useful and meaningful fractionation (Melville et al., 1969, Freney et al., 1969). Firstly, chelating resins

were examined but these only extracted 44 percent of the total organic sulphur and their ability to extract this amount was due to the high pH of the resin rather than chelation properties. These resins had been previously used to extract unaltered organic matter from soils, but were clearly limited when used in connection with sulphur studies. Freney et al., (1969) examined a wide range of compounds for extracting organic sulphur, but although they found that no compound tested was entirely satisfactory, a mixture of sodium carbonate and sodium bicarbonate proved the most effective. The other soil organic matter extractents examined were, sodium hydroxide, chelating resins and sodium pyrophosphate. Only sodium hydroxide extracted more than 50 percent of the organic sulphur but the nitrogen:sulphur ratio of the material extracted differed from that of the soil. Therefore no reagent successfully extracted a representative sample of the organic matter. It was also found that very alkaline conditions caused breakdown of humic acid sulphur compounds to fulvic acid sulphur. Although this work by Freney et al., (1969) was originally designed to find a suitable extractant for soil organic sulphur, several additional pieces of information were obtained. For example, results suggested that much of the HI-reducible sulphur occurred as high molecular weight humic acid compounds and not as fulvic acid compounds as often previously suggested. Freney (1961) used sodium hydroxide to extract soil sulphur which probably caused

humic sulphur to be broken down into fulvic sulphur. Another interesting finding of the work was the detection of very little inorganic sulphur in the extracts although on storage at high pH values organic sulphur was converted to inorganic sulphur. This could possibly be used as a measure of labile sulphur organically combined in the soil. Yet another point concerned the increased HI-reducible sulphur observed on storage at high pH, indicating that C-S bonds were broken, as would occur with proteins and peptides containing cysteine (Freney, 1967).

Sodium hydroxide/sodium pyrophosphate (pH 13) has also been employed to extract soil sulphur (Bettany et al., 1979 and 1980). The extracted sulphur was then separated into humic acid sulphur (HA-A), fulvic acid sulphur (FA-A), clay-associated humic acid sulphur (HA-B) and humin ($< 2\ \mu m$) sulphur. The relative amounts of HI-reducible sulphur and carbon-bonded sulphur were then determined for each sulphur fraction. On the basis of lower C:N:S ratios and higher proportions of HI-reducible sulphur in the FA-A, HA-B and humic fractions it was suggested that these three fractions contained the major soil reserves of potentially labile sulphur.

Recently an extractant has been examined which removes 61-97 percent of soil organic sulphur (Scott and Anderson, 1976). An advantage is that the extractant, acetylacetone, extracts under very mild chemical conditions and is therefore likely to extract unaltered forms of soil organic sulphur. The extract was fractionated using gel

permeation chromatography and although four distinct fractions were obtained, there was no consistent similarity between the corresponding fractions of different soils. However, such an efficient extractant operating under mild conditions does provide a new starting point for further research into soil organic sulphur.

2.2.4 Characterisation of soil organic sulphur fractions.

Since no, new, better, extraction procedures could be readily developed, another approach was to persist with the widely used HI-reducible/carbon-bonded sulphur fractionation and try to more fully characterise these fractions. Whilst some information is available on the metabolism of the carbon-bonded sulphur compounds, methionine and cysteine, (Frederick et al., 1957, Freney, 1961) very little information is available on the HI-reducible fraction. It was not known whether the HI-reducible sulphur was a relatively stable end product or an active participant in sulphur metabolism (for example, an early stage of incorporation of inorganic sulphur into organic sulphur). An attempt to further characterise these fractions was undertaken by Freney et al., (1971) where labelled inorganic sulphate was added to soils which were then incubated. Incorporation of sulphur-35 into the two fractions was subsequently measured. The results of this work showed no consistent pattern of sulphate incorporation; the labelled sulphate being randomly incorporated into both fractions. This suggested that the fractionation procedure had little relevance to soil

organic sulphur transformations. Freney *et al.*, (1971) recognised the severe limitations imposed by this fractionation; "it is clear that more detailed subdivision of the HI-reducible fraction will be necessary before the part played by it in sulphate incorporation can be understood." This work did show, however, that most of the labelled sulphate was incorporated into the fulvic acid fraction after 24 weeks incubation, but this could be due to the short period of investigation, i.e., given more time fulvic sulphur could be converted to humic sulphur. Also sodium hydroxide was found to extract the same ratio of labelled:unlabelled sulphur as occurred in the soil after incubation, suggesting that this reagent extracts a representative sample of organic matter even though the extracted compounds may not remain entirely unaltered.

Another method of more fully characterising the organic sulphur fractions was adopted by Freney *et al.*, (1975) where the fractions were labelled with sulphur-35 and assessed as sources of plant available sulphur. This approach contrasted with the method of above, as this time mineralisation rather than incorporation (immobilisation) was studied. Here again, however, results did little to justify continued use of the HI-reducible/carbon-bonded sulphur fractionation. Experiments were set up which enabled the determination and isotopic assay of organic sulphur fractions, both before and after the growth of Sorghum. The sulphur taken up by the plants was found to be almost equally derived from both fractions although

slightly more carbon-bonded sulphur was taken up. It is very interesting to note that some transformation of the carbon-bonded sulphur (which is not reducible by Raney nickel) occurred. Thus it seems that earlier work (Lowe, 1965) which postulated the existence of a small, inert, plant insignificant fraction of organic sulphur was incorrect and that the alternative theory (Freney et al., 1970), that Raney nickel was incapable of extracting all carbon-bonded sulphur, is more acceptable. Since all the sulphur fractions investigated contributed to the pool of available sulphur "none of them are likely to be of any value for predicting the sulphur requirements of plants" (Freney et al., 1975). Thus, it seems that these fractions, which are based on purely chemical properties of the sulphur compounds bear little relationship to sulphur compounds which are either readily mineralised or which are the products of microbial incorporation of inorganic sulphur (immobilisation). Goh and Tsuji (1979) and Tsuji and Goh (1979) adopted a similar approach to characterise soil sulphur fractions. Soils were incubated with S-35 labelled gypsum for 70 days. During the incubation sub-samples were periodically taken and extracted with various reagents. Up to 80 percent of the applied sulphur was immobilised whilst indigenous sulphur was simultaneously mineralised. However the high specific activity of the inorganic sulphur was not distinguished from the lower specific activity of the organic HI-reducible sulphur and therefore invalid assumptions were made regarding the

specific activity of the organic sulphur pool. The soils, labelled with S-35 (the S-35 was not equilibrated with indigenous sulphur) were then sown with ryegrass. Amounts of sulphur taken up were correlated with decreases in extractable sulphur. $Ca(H_2PO_4)_2\ 2H_2O$ and NaH_2PO_4-HOAC proved to be the best correlated extractants.

Other attempts to characterise the soil organic sulphur fractions have involved comparing the fractions of a cultivated soil with those of a pasture soil (McLaren and Swift, 1977). Both soils had been maintained in the aforementioned conditions for many years and both belonged to the same soil series (therefore mode of agricultural practice employed was the only essential variation). The cultivated soil contained less total sulphur due to the mineralisation of sulphur stimulated by long term cultivation. Although 75 percent of the sulphur lost came from the carbon-bonded sulphur pool (and therefore only 25 percent from the HI-reducible sulphur) the authors suggested that the HI-reducible sulphur pool was more transitory in nature and hence assumed greater importance in short term mineralisation. The following chain of events was therefore proposed (McLaren and Swift, 1977):-

$$\text{C-S} \rightarrow \text{HI-reducible S} \xrightarrow{\text{mineralisation}} \text{inorganic S.}$$

The findings of this work have been corroborated by recent work in Canada (Bettany *et al.*, 1980) which also compared permanent pasture and continuously cultivated soils. This work indicated that the traditional fractionation scheme

had some use in predicting the sulphur supplying power of soils which is in contrast to earlier findings (Freney *et al.*, 1975). McLachlan and De Marco (1975) noted that accumulation of added sulphur to a pasture soil occurred in the carbon-bonded fraction. However subsequent cropping showed that this carbon bonded sulphur was taken up by plants and a similar carbon-bonded sulphur $\leftrightharpoons$ HI-reducible sulphur conversion was postulated.

2.2.5 Soil organic sulphur in U.K. soils

Little work has been published about the organic sulphur fractions of British soils. However some work on Scottish soils has been performed (Scott and Anderson, 1976b. It was found that calcareous soils contained proportionally less HI-reducible sulphur than acid soils, although no reasoning could be offered for this observation. This Scottish work was significant in that further evidence was obtained to support the HI-reducible/carbon-bonded sulphur separation. This was that the Raney nickel-reducible sulphur was well correlated with total carbon, total N and organic sulphur "suggesting that a well defined group of compounds was being measured". However, "well defined" may only be applicable in a chemical sense with the compounds being ill defined in terms of soil sulphur mineralisation or immobilisation.

In summary, although the soil organic sulphur fraction is vitally important in the long term maintenance of plant available sulphur supplies, very little is known of its

composition, either from a chemical or from a transformation point of view. The HI-reducible/carbon-bonded sulphur fractionation has been widely employed and is possibly answerable for the slow advancement of our understanding. The criteria on which the separation is based has shown questionable relevance to processes operative in soil sulphur cycling. Until a more satisfactory separation is developed (or at least a subdivision of the existing fractions) our knowledge of soil organic sulphur remains limited.

2.3 The Mineralisation of Soil Sulphur

It is generally accepted that for the soils of the humid regions practically all soil sulphur occurs as organic forms. Since plants take up sulphur as sulphate or as small uncombined organic molecules, such as cystine and methionine (Bardsley, 1960), the large complex molecules in soil organic matter must undergo degradation into plant available forms, if plants are to remain sulphur sufficient. Although mineralisation of soil sulphur is important in the long term sulphur status of soils, the amounts produced rarely match plant requirements. Hence in areas where mineralisation is almost the sole supplier of available sulphur, deficiencies occur. Such areas are usually non-industrialised, inland and contain only small amounts of indigenous mineral sulphur, e.g. France, New Zealand, Australia and south-east U.S.A. (Coleman, 1966).

2.3.1 Methods used to study the mineralisation of soil sulphur.

It is believed that mineralisation of sulphur is predominantly brought about by the soil microflora and therefore a system of mineralisation/immobilisation would be expected to occur (Freney and Stevenson, 1966). Therefore sulphur mineralisation is a very difficult process to investigate, owing to the vast range of types of interacting micro-organisms which occur in soils. Cooper (1971) made such an observation and suggested that incubation studies, where initial and final amounts of sulphate sulphur could be determined, would be most relevant and useful. These incubation methods are excellent for demonstrating the effect of a treatment on the process but do little to help elucidate biochemical pathways (i.e. the actual working mechanism of the process). Thus it follows that our overall knowledge of organic sulphur mineralisation is extremely limited especially with regard to biochemical pathways.

2.3.2 Factors affecting the mineralisation of soil sulphur

Most of the work in recent years on sulphur mineralisation has been performed in Australasia and North America, usually by means of incubation studies. Spencer and Freney (1960) found that only 2 μgS/g of sulphur was mineralised during an incubation period of 14 months, which is very little. However, this figure is only based on a sulphur balance sheet and the 2 μgS/g represents net

sulphur mineralisation. The same workers also found a correlation between mineralised sulphur and phosphate extractable sulphur, a correlation which does not comply with current knowledge that phosphate solutions extract easily soluble, absorbed and some organic sulphur. Also in 1960 Freney and Spencer noticed an effect on mineralisation rates due to the presence of growing plants, more sulphate being mineralised where plants were present (this effect has also been noted by recent workers, Tsuji and Goh (1979)). This was considered to be due to the increased microbial activity of the rhizosphere and showed that plants influence the rate of sulphur cycling in the soil. Barrow (1961) performed a detailed study of sulphur mineralisation and found that air drying of soils caused a flush of sulphur mineralised upon subsequent incubation which could be due either to the splitting up of organic molecules on drying, or more likely to the Birch effect where drying kills many microflora and thus provides a source of easily assimilable substrate (hence increased microfloral activity results). Barrow also found that the addition of plant material with low sulphur content caused a net immobilisation of sulphate and that water extractable sulphate over-estimated amounts of sulphate which were available to micro-organisms. This led to a preference for 0.15 percent $CaCl_2$ as an extractant for mineralised sulphur. The amount of sulphur extracted by 0.15 percent $CaCl_2$ was later found to correlate with microfloral activity, as measured by carbon dioxide evolution

(Kowalenko and Lowe, 1975). Work on Mississippi soils (Nelson, 1964) revealed a common pattern of sulphate release with time, the soils showed variable release or fixation for 2 months but then mineralisation proceeded at a steady rate. This initial variable release was probably due to differing C:N:S ratios, an average ratio being 126:10:1 which is similar to that reported for a range of New Zealand soils (White, 1959).

Liming has been shown by several workers (Nelson 1964, White 1959 and Williams 1967), to affect the rate of sulphur mineralisation. Nelson (1964) demonstrated that soils of pH 5 or less when limed to pH 6.5 contained three times more ammonium acetate/acetic acid soluble sulphur which was attributed to an increased rate of mineralisation. In view of desorption evidence (see section 2.4) the increased sulphur extracted was possibly due to the decreased adsorption capacity of the soil for sulphur as a result of increased pH. Freney and Stevenson (1966) have offered other possible explanations of the liming effect, which are; sulphate added in the lime, sulphate released by chemical hydrolysis at the higher pH or increased mineralisation due to optimisation of the pH environment. Toluene and formaldehyde were found to suppress mineralisation but the effect was less marked on addition of calcium carbonate (Williams 1967) suggesting that microfloral activity was stimulated, although again chemical effects cannot be eliminated.

Since it is generally believed that sulphur mineralisation occurs as a result of microfloral activity

it is not surprising to find that the conditions which affect microfloral activity also affect mineralisation rates as measured by incubation. Thus Williams (1967) found that soil temperature and soil water content affected sulphur mineralisation. The optimum temperature was approximately 30°C with a marked fall off in mineralisation exhibited at 10°C and an intermediate effect at 20°C. Similarly, low moisture content (10 percent) and water-logging decreased the amounts of sulphur mineralised with time. The effects of added nitrogen (in various chemical forms) on net sulphur mineralisation in two Canadian soils has been studied (Kowalenko and Lowe, 1978). The two soils exhibited contrasting results although significant interactions between soil, rate of N applied and chemical form of the sulphur were obtained. These findings indicated that simple causal relationships between applied N and mineralised sulphur cannot be made.

As already briefly mentioned, the sulphur content of added plant material (e.g. manures and plant residues) drastically affected the available sulphur supply by mineralisation or immobilisation of sulphur. Stewart et al., (1966) suggested a critical value of 0.15 percent sulphur. Thus organic additions with a sulphur content below 0.15 percent will result in immobilisation of soil sulphate. This subject is especially relevant to arable soils receiving large N,P,K additions since a widening of the ratio of sulphur to other plant nutrients favours sulphur immobilisation processes.

Several workers have compared the rates of nitrogen and sulphur mineralisation, but results have varied widely. Stewart and Whitfield (1965) state that nitrogen and sulphur are probably mineralised in the same ratio as they occur in the soil organic matter, a hypothesis supported by the results of Nelson (1964). However Haque and Walmsley (1972) found that for West Indian soils nitrogen and sulphur were never released in the ratio existing in the organic matter. Several other reports of this disparity exist [Barrow, (1961), Swift (1977), White (1959)]. This change in the nitrogen:sulphur ratio could be due to the two elements occurring in different fractions of the organic matter (Freney and Stevenson, 1966) or to air drying differentially affecting subsequent mineralisation of each element. Recent work by Kowalenko and Lowe (1975) indicated that complex relationships between C:N:S ratios and sulphur mineralisation exist and that the inter-relationship of C:N:S is a vital consideration when assessing the sulphate supplying power of the soil. Other workers however, (Swift, 1977) do not place so much importance on elemental ratios. Swift (1977) noted that amounts of sulphate mineralised did not correlate with C:S or N:S ratios. In addition sulphur was found to be less mineralisable than either carbon or nitrogen. By examining C:N:S ratios of pairs of permanent pasture soils and cultivated soils of the same soil series it was shown that the C:S ratio and the N:S ratio was significantly lower in the arable soils. This means that long term

cultivation has brought about a relative enrichment of sulphur in the soil organic matter and additionally showed that sulphur was less susceptible to mineralisation than either C or N. The results obtained for seven pairs of soils suggested that total sulphur would provide the best indication of a soils ability to mineralise sulphur.

Very little is known of which fraction of organic sulphur is most susceptible to mineralisation, but losses in the HI-reducible fraction have been correlated with sulphate produced during mineralisation (Cooper, 1971). Bettany et al., (1974) were however unable to correlate mineralised sulphur with total sulphur, HI-reducible sulphur or percent of total sulphur occurring as HI-reducible sulphur. This illustrates once again that the existing fractionation of soil organic sulphur is not exactly related to transformations in the soil.

The fact that air drying of soil samples, prior to incubation studies, increases rates of sulphur mineralisation has been noted by many workers (Kowalenko and Lowe, 1975, Williams, 1967 and Barrow, 1961) but no satisfactory reasoning has been given. It has always been considered to be due to the Birch effect, but it has been shown that air drying introduces sulphur transformations which are unrelated to microbial activity as measured by carbon dioxide evolution (Kowalenko and Lowe, 1975). Thus it seems that chemical or physical agents are probably responsible for the larger amounts of sulphate extracted from dried soils. It is interesting to note that

Williams (1967) was unable to observe a flush of sulphur mineralisation in soils which had dried out naturally in the field over summer, whereas the corresponding soils air dried in the laboratory, exhibited a marked flush of sulphate production on incubation. An explanation for this could be that field drying was less severe which would lessen the Birch and physico-chemical effects. Barrow (1961) found that severity of drying was directly proportional to the quantity of sulphate subsequently extracted.

2.3.3 The role of arylsulphatase in soil sulphur mineralisation.

In addition to the microbial mineralisation of sulphur, soil enzymes possibly convert significant amounts of organic sulphur into available forms. Interest in this possibility began when Tabatabai and Bremner (1970) first reported the presence of arylsulphatases in Iowa soils. These enzymes had been previously widely reported as occurring in plant and animal tissue, but never before in soils. The hydrolysis of aryl sulphates by these enzymes can be represented by the following reaction:-

$$RO\ SO_3^- + H_2O \rightarrow ROH + H^+ + SO_4^{2-}$$

A method for the determination of arylsulphatase activity in soils (Tabatabai and Bremner, 1970) was based on the colorimetric estimation of p-nitrophenol which is released on enzymatic hydrolysis of potassium nitrophenyl sulphate during incubation. The same workers also

investigated factors affecting arylsulphatase activity and found that activity decreased with profile depth to a similar degree with which organic matter also decreased. However, no correlation between activity and pH, total sulphur, total N, % sand or % clay was observed. Air drying of soils caused a significant rise in activity, an effect also observed with other soil enzymes (Bremner, 1965). Oven drying at 105°C caused a marked decrease in activity, presumably due to a denaturing effect of heat on the enzymatic protein. Arylsulphatase has also been studied in Nigerian soils (Cooper, 1971) and activity was found to correlate with total carbon, total organic sulphur and especially HI-reducible sulphur. These observations comply with existing knowledge of the HI-reducible sulphur as a fraction consisting of compounds in which sulphur is not directly linked to carbon - a suitable substrate for arylsulphatases. It was also observed that wetting and drying cycles reduced activity and it was concluded that although activity was correlated with soil properties seasonal variations might be dominant in influencing activity (however these climatic variations are particularly severe in Nigeria, where this work was performed). The more recent work completed on this aspect of sulphur mineralisation suggests that arylsulphatase could be much less significant than previously believed. Kowalenko and Lowe (1975) noted that activity declined sharply during a 14 month incubation period although activity was correlated with 0.15 percent $CaCl_2$

extractable sulphur. Lee and Speir (1979) compared the uptake of organic sulphur from twelve soils by perennial ryegrass with sulphatase activity. Their findings showed a significant correlation between sulphatase activity and organic sulphur uptake although an organic sulphur rich soil strongly influenced the correlation obtained. The workers stress that since sulphatase is likely to be only one of many enzymes operational during sulphur mineralisation good correlation is unlikely. Indeed biochemists have isolated a wide range of sulphohydrolases from soils (Houghton and Rose, 1976). Therefore the significance of soil enzymes in the mineralisation process remains unclear.

Another approach to the study of soil sulphur mineralisation involves the use of pure microbial colonies, such methods have been extensively reviewed (Freney, Barrow and Spencer, 1962; Whitehead, 1964; Freney and Stevenson, 1966; Freney, 1967 and Cooper 1971) and, since little current work is being carried out, will not be reviewed here. Most of the work has been carried out using cystine and methionine as substrates probably because of their importance in plant and microbial metabolism. Pure culture work is limited as extrapolation of results to the soil system is uncertain and hence little is known of the biochemical pathways associated with mineralisation of soil organic sulphur.

To summarise, mineralisation of sulphur compounds is almost entirely due to the activities of the microflora but

soil enzymes along with physical and chemical effects cannot be ignored. Since microbial activity probably predominates, rates of mineralisation will be affected by factors affecting microbial activity, e.g. temperature, water content and pH. The rate of mineralisation is also dependent on the nature and chemical composition of the substrate, the sulphur content and C:N:S ratio of the substrate being of particular importance in deciding relative rates of mineralisation and immobilisation.

2.4 The Retention and Movement of Soil Sulphur

Sulphate is the form of sulphur which is most susceptible to leaching due to its solubility and negative charge. Since this is also the form of sulphur taken up by plants, sulphate retention by the soil improves the sulphur status of soils. In the vast majority of soils the inorganic sulphur occurs almost exclusively as sulphate. For example Freney (1961) showed that less than 1 percent of the total soil sulphur occurred in inorganic compounds less oxidised than sulphate. Low sulphate levels have been frequently found in surface horizons of soils. This observation is prevalent in agricultural soils of humid regions since they are very prone to leaching (Ensminger, 1954; Freney, 1961; White, 1959). It is because of these low sulphate levels that replenishment by organically combined sulphur by mineralisation is so important, especially where atmospheric inputs of sulphur are very low. Soils of the more arid regions frequently contain sulphur precipitated as calcium sulphate (gypsum)

which can accumulate in the profile, often as a pan. Some insoluble sulphates have been found in the soil such as barytes (Cairns and Richter, 1960; Williams and Steinberg s, 1962) but the vast majority of sulphates are water soluble.

From the above introductory paragraph it is clear that sulphate retentive soils offer a distinct advantage to the sulphur nutrition of the plant. Several questions arise:

(a) is the adsorbed sulphate plant available?

(b) how strongly bound and by what means is the sulphur retained?

(c) which soil constituents are most important in retaining sulphate?

(d) can the management of soil influence the capacity for sulphate retention?

2.4.1 The soil constituents responsible for sulphate adsorption

Early work (Ensminger, 1954) found that Alabama surface soils showed little capacity for sulphate retention, whereas most subsoils could retain some sulphate against leaching. Several workers (Kamprath *et al.*, 1956, Hasan *et al.*, 1970 and Haque and Walmsley, 1973) noticed that intensely weathered soils, such as those found in the tropics, were capable of considerable sulphate retention. This type of soil contains kaolinite, and iron and aluminium hydroxides which are typical products of intensive weathering. Ensminger (1954) found that aluminium oxide retained much more sulphate than did iron oxides while kaolinite and bauxite showed intermediate effects.

Kaolinite has been studied as a possible soil constituent responsible for sulphate retention (Ensminger, 1954; Kamprath et al., 1956; Aylmore et al., 1967; Chao et al., 1962c.) Since the adsorption of sulphate onto Kaolinite has been shown to be completely reversible (Aylmore et al., 1967) the adsorbed sulphate is probably available to plants. The same group of workers also found that pH affected sulphate adsorption onto kaolinite, with adsorption decreasing with increasing pH. This complies with existing knowledge (Graham and Thomas, 1947) that 1:1 clay minerals have a pH dependent charge with positively charged sites formed by the hydration of hydroxy groups at lower pH values. The positive sites thus developed are then electrostatically balanced by anions. Organic matter has also been investigated as a possible retainer of sulphate (Chao et al., 1962c.) Known retentive soils were studied before and after the removal of organic matter. It was found that on removing the organic matter the ability of the soil to retain sulphate was reduced by half. However, earlier work (Chao et al., 1962a) showed that some very organic soils had almost no retention capacity. Therefore, the contribution of organic matter is unclear particularly since the hydrogen peroxide oxidation step used to remove the organic matter, could have reduced the capacity of some other soil components to retain sulphate. Alternatively, the types of organic matter associated with different soils may have differing abilities to retain sulphate.

Evidence is available for the adsorption of sulphate by iron and aluminium oxides. This has been demonstrated by constructing adsorption isotherms before and after the removal of sesquioxides from soils, or by examining laboratory manufactured oxides (Aylmore et al., 1967). De-aluminated soils sorbed much less sulphate and deferrated soils sorbed even less. (Chao et al., 1962c) However doubts are expressed about the amounts of sulphate removed and the specificity of the extracting reagents. Aylmore et al; (1967) performed adsorption and desorption studies on pure samples of haematite and pseudoboehmite (γ Al_2O_3 H_2O) and found that Langmuir relationships were obeyed and that adsorption maxima were directly correlated with specific surface area. This indicated that similar adsorption mechanisms which related to surface area were in operation. It was additionally found that the process of adsorption was completely irreversible with no desorption from an admixture of iron oxide and sand after leaching with the equivalent of 20 inches of rain. The workers suggested that the sulphate could be unavailable to plants.

2.4.2 Mechanisms proposed to explain sulphate adsorption by soils

Several workers have postulated sulphate adsorption mechanisms for iron and aluminium minerals and 1:1 clay minerals. However the present literature offers many conflicting views (Chang and Thomas, 1963; Chao et al., 1962b) Chao et al., 1963; Aylmore et al., 1967). The

adsorption isotherm for kaolinitic minerals shows two distinct adsorption regions, which suggests that two energetically different sites are available for the adsorption of sulphate (Aylmore et al., 1967). Since the order of adsorption of sulphate onto H^+ saturated clay minerals is kaolinite > illite > bentonite, (this order bears no relationship to surface area) clay structure must be influencing adsorption. The form of the aluminium on the clay crystal surfaces was altered by adjusting the pH of the bathing solution and adsorption isotherms were constructed (Chao et al., 1962c). Results were compared with an H^+ exchange resin. It was found that the resin adsorbed more sulphate with increased pH, yet the clay minerals adsorbed less with increased pH. This indicated that the positive sites created at low pH on the clays were more important than the amphoteric aluminium complexes formed at higher pH values. Hydroxyl exchange sites were formed on the H^+ resin. After extensive work by Chao et al., (1962c) the following sulphate adsorption mechanisms were suggested:-

(a) Anion exchange at the positive sites on hydrous iron and aluminium or 1:1 minerals at low pH values.

(b) Co-ordination complexes between sulphate and hydroxy aluminium complexes.

(c) Salt adsorption (the simultaneous adsorption of cation and anion regardless of the forces involved).

(d) Anion exchange under specific conditions when the amphoteric property of organic matter gives rise to

positive sites. This anion exchange theory was later tested (Chao et al., 1965) by the same workers who made use of the fact that a pH rise accompanied the adsorption of sulphate if the anion exchange model (see mass action equation below) applied.

$$\text{Soil} \begin{matrix} OH \\ OH \end{matrix} + SO_4^{2-} \rightleftharpoons \text{Soil} \quad SO_4 + 2OH^-$$

The workers found an increase in pH after sulphate adsorption onto retentive soils. This increase was more pronounced when the soil was artificially coated with iron oxide and when the SO_4^{2-} concentration in the liquid or the mass of soil used was increased. Therefore they concluded that pH increase represented replacement of OH^- with sulphate ions. However, Chang and Thomas (1963) found no such similar pH change to substantiate the $2\ OH^-/SO_4^{2-}$ exchange model. Chao et al., (1963) reported the effect of cations on sulphate adsorption by soils. The amount of sulphate adsorbed, following saturation of the soil with a cation was in the order $Al^{3+} > Ca^{2+} > K^+$ (a decreasing order of valency).

The influence of cations was probably due to their effect on pH but a definite cation effect was found, possibly attributable to their effects on zeta potentials and anion repulsion.

2.4.3 Sulphate adsorption isotherms

Several workers have constructed adsorption isotherms, for sulphate on soil (Chao et al., 1962b,c; Hasan et al., 1970; Fang et al., 1962; Haque and Walmsley, 1973).

Chao et al., (1962b) equilibrated soil with 5, 10, 15, 20, 25 and 30 µg/g S and found that the sulphate adsorbed increased linearly with concentration in the equilibrium solution (up to 25 µgS/g). If adsorption was solely due to anion adsorption one would expect an adsorption maximum (c.f. cation exchange capacity). Therefore 100, 200, 300, 400 and 500 µgSO_4^{2-}-S/g solutions were equilibrated with a soil of known retentive qualities, but no adsorption maximum was found (therefore no definite anion exchange capacity existed). Only the data for 0 → 25 µgSO_4^{2-}-S/g, obeyed a Langmuir plot. Where greater concentrations of sulphate had been equilibrated the Freundlich relationship was observed. This again indicated no adsorption maximum, and did little to elucidate possible adsorption mechanisms. Using a chromatographic method, Fang et al., (1962) found that Freundlich type equations held for weakly adsorbing soils. The isotherms obtained suggested that two different sites of adsorption, each in equilibrium with the other, existed. Haque and Walmsley (1973) found that the Langmuir equation was followed up to 100 µgS/g of added sulphate when a "1st phase adsorption maximum" was reached. Beyond this sulphate concentration other sites with a different bonding energy might become important. Hasan et al., (1970) adsorbed sulphate onto soils at low sulphate concentrations and found a linear relationship between log sulphate concentration in soil solution and sulphate sorbed or desorbed (no hysteris effects were reported). This

isotherm is very useful for predicting the capacity and intensity of the soil to supply available sulphur to plants. Highly weathered soils possessed a high potential buffer capacity (steeply sloped isotherm). This linear relationship only held up to 30 $\mu g SO_4^{2-}$ S/g concentration and a "first phase" adsorption maximum could be calculated. The same workers showed, using pot studies, that poorly adsorbing soils needed higher (> 20 μg S/g) soil solution concentrations of sulphate than the more retentive soils where only 5 μgS/g was sufficient to prevent plant deficiencies.

2.4.4 Desorption of sulphate in soils

Interest has been shown in the desorption of sulphate since the ease of desorption affects the availability of sulphate to plants and governs amounts lost by leaching. Several workers have noted that applications of phosphate decreased the adsorption capacity of the soil for sulphate (Ensminger, 1954; Kamprath et al., 1956; Chao et al., 1962a) due to a preference for the phosphate anion at the exchange sites. These workers also noted that liming increased the sulphate concentration in the leachates and reduced the sulphate adsorption capacity of the soil (due to a loss of positive charges on 1:1 type clay minerals and sesquioxides). The ease of sulphate desorption seems to vary greatly with soil type. For example, Hasan et al., (1972) working with Hawaiian soils found complete reversibility of sulphate adsorption. Aylmore et al., (1967) reported complete irreversibility when considering

pure pseudoboehmite and haematite. This contradiction was somewhat resolved by later work (Sanders and Tinker, 1975) which showed that where soil sulphate retention properties were due to the presence of haematite, completely reversible adsorption/desorption was observed. However, a study of the isolated haematite showed completely irreversible sulphate adsorption, indicating that weathered sesquioxides show little hysteresis between adsorption and desorption. This situation illustrates the problems of using pure, manufactured soil components and extrapolating back to the soil system.

Barrow (1967) found that the decrease in extractable sulphate (which included some adsorbed sulphate) exactly balanced plant uptake as measured in a pot experiment. This indicated that adsorbed sulphate was plant available. Such a conclusion has been reached by other workers (Elkins and Ensminger, 1971). Plant availability of sulphate was used as a measure of the change in strength of sulphate adsorption with time (Barrow and Shaw, 1977). The work showed that sulphate became more strongly bonded over a period of six months as indicated by desorption techniques, sulphate concentration in soil solution and plant availability.

2.4.5 Leaching losses of sulphur from soils.

The study of sulphate leaching from soils is fraught with experimental difficulties and problems of data interpretation. Movement of sulphur down packed soil columns has been monitored by using sulphur-35 (Chao et

al., 1962a). These leaching experiments were designed to elucidate adsorption mechanisms but were discarded in favour of equilibration techniques. As expected, increased addition of water increased the movement of sulphate down the column. However when the addition of sulphate was increased, and only one quantity of water applied, the pattern of movement remained the same suggesting that retained sulphate was in kinetic equilibrium with sulphate in solution. The degree of movement depended greatly on soil texture and in the field, soil structure would also be expected to influence movement. To accomodate the soil structural component undisturbed cores have been leached with water in the laboratory (Peverill and Douglas, 1976). Results indicated that up to 17 kgS/ha/yr could be leached from an unamended sandy podzol. The monitoring of sulphate movement in the soil has inherent practical difficulties as found by Till and McCabe (1976) who employed lysimeters in conjunction with sulphate-35. Their findings indicated small leaching losses of sulphate from grazed pasture soil despite apparent total losses of 23 kgS/ha/yr. However the workers stress the limitations of data drawn from lysimeter studies (due to uneven flow rates and edge effects). Hogg and Toxopeus (1966) discovered appreciable losses of applied sulphate (as superphosphate), within four weeks of application, which amounted to 33-100 percent of that applied. This is in sharp contrast to the lysimeter results. Gregg et al., (1977) monitored the movement of

sulphate-35 in the field and noted the effect of soil type on sulphate movement. Bailey (1974) examined the distribution of applied sulphate (as superphosphate) two years after application. The lower addition of sulphate (67 kgS/ha) showed accumulation of sulphate at 40-50 cm depth despite 37 kgS/ha being removed by plants and an overall loss from the soil profile of 14 kgS/ha. Overall sulphate losses varied from 14-41 kgS/ha over two years.

2.4.6 Sulphur lost from soil by volatilisation

Another topic which can be broadly classed under the heading of sulphur movement is the loss of sulphur from soils by volatilisation. When studies of sulphur mineralisation are performed the sulphur present at the outset can usually be recovered after the period of incubation, but Nicholson (1970) found a net loss of sulphur which could not be attributed to analytical error. It was postulated that these losses could be due to sulphur volatilisation caused by micro-organisms. In the same year independent work was carried out on the evolution of volatile sulphur compounds from decomposing crucifers (cabbage, kale, mustard for example) in soil (Lewis and Papavizas, 1970). Such compounds as dimethyl sulphide, methanethiol and dimethyl disulphide were detected by gas chromatography and chemical analysis. However, no similar compounds were detected when corn residues were incubated with soil. This work indicates that sulphur volatilisation might only be significant under very specific conditions. The lack of an accurate

and sensitive analytical procedure for detecting volatile sulphur compounds hindered work in this field, until Bremner and Banwart(1974) developed a suitable gas chromatographic technique. It was found that volatile sulphur compounds were liberated from soils incubated with methionine and cystine (Banwart and Bremner, 1976a). Later, (Banwart and Bremner, 1976b) both unamended and sulphate treated soils were examined. Results showed that only four out of twenty-five aerobic Iowa surface soils liberated volatile sulphur compounds. The amounts liberated only represented tiny fractions of the total soil sulphur content. Water-logged soils were also studied, but again losses by volatilisation were insignificant. Nearly all (90%) of the volatilised sulphur was identified as dimethyl sulphide although traces of carbonyl sulphide, methyl mercaptan and dimethyl disulphide were found. The workers concluded that gaseous losses of sulphur from soil were insignificant. Other workers have detected the emission of dimethylsulphide from agricultural soils (Farwell et al, 1979). There could be more sulphur volatilisation occurring in soils than was indicated by the measurement of volatile sulphur compounds in the free atmosphere above the soil, as soils can adsorb volatile sulphur compounds (Bremner and Banwart, 1976a). Both air-dried and moist soils sorb dimethyl sulphide and dimethyl-disulphide although the capacity for adsorption is much lower than for sulphur dioxide and methyl mercaptan. It was thought that the

microbial population of the soil was partly responsible for the sorption and the work showed that the soil was a large potential sink for environmental pollutants. The significance of sulphur lost by volatilisation remains unclear and further investigation on a wider range of soil types is needed.

To summarise; the movement, reactions and adsorption of sulphur occurs predominantly as sulphate. Thus, it is vital for the soil to have an adequate ready supply and sufficient reservoir of sulphate in the rooting zone. The largest reservoir of sulphur is organic matter, but where this is small or mineralisation rates are low the importance of adsorbed sulphate increases, especially in humid agricultural soils which contain very low concentrations of sulphate in the soil solution. Thus, adsorbed sulphate is an important source of plant available sulphur, the quantity of which depends on the mineral composition of the soil, soil pH, soil management practises (such as liming and phosphatic fertilisation) and rates of leaching.

2.5 The Assessment of Soil Sulphur Status

The evolution of methods used to assess soil sulphur status has been similar to that for nitrogen. This is because the major reserve of both elements is the soil organic matter. Methods examined have included extraction of soil sulphur by various solvents, incubation of soil samples, assay based on microbial growth, plant

growth and composition. Results are then statistically analysed with the aim of establishing a positive, significant correlation between, for example, nutrient uptake and nutrient extracted. It was soon realised that the measurement of total sulphur was quite inadequate as an index of available sulphur (Bardsley and Lancaster, 1960). In light of present knowledge this would be expected since the proportions of unavailable and available sulphur were not determined. Since total sulphur showed no promise, emphasis was placed on the use of extractants and subsequent measurement of sulphur extracted. Here again problems existed, for instance it was soon found that sample preparation greatly affected the amount of sulphur extracted by one particular extractant. Air drying was found to increase amounts of extractable sulphur (Barrow, 1961; Freney, 1958; Williams and Steinbergs, 1959) but even now no standardised procedure for the collection and preparation of soil samples has been established.

Early work (Williams and Steinbergs, 1959) had failed to find an extractant (0.1N sodium hydroxide, HI-reducible sulphate were tried) which removed quantities of sulphur which correlated with yields and led these workers to believe that some labile organic fraction might be usefully measured. Thus, "heat soluble sulphur" was developed which measured water soluble sulphate after boiling soil samples in water for one hour. This increased sulphate release was believed to be a measure of

the easily mineralisable or labile soil sulphur and correlated better with plant yields. Subsequent work, however, has tended to ignore this method.

A great variety of extractants have been examined, but since our knowledge of the nature of soil sulphur is limited such methods are rather haphazard since various proportions of unknown fractions of soil sulphur are extracted. Therefore, an extractant which might prove useful for a soil containing much adsorbed sulphate might be totally inadequate for a soil where most of the sulphur occurs in the organic fraction. Such extraction procedures are still being examined but will not cease to be empirical until soil sulphur fractions are isolated and characterised. However, such empirical approaches have, to some extent, been useful in estimating the soils' ability to provide available sulphur.

Kilmer and Nearpass (1960) found that the best measure of available sulphur was the soil sulphur extracted by 0.5 M sodium bicarbonate (adjusted to pH 8.5). Although the workers were unsure of the forms of sulphur extracted, good correlation between extractable sulphur and sulphur "A" values was obtained, ("A" values are obtained in the greenhouse and refer to the uptake of soil applied sulphur-35). The extracted soil sulphur was measured by the method of Johnson and Nishita (1952), and therefore HI-reducible organic sulphur forms, in addition to extracted sulphate were measured. It is likely that the 0.5M $NaHCO_3$ at pH 8.5 removed organically combined and

adsorbed sulphate which could account for the good correlation obtained above (especially if the extracted organic sulphur was readily mineralisable).

In Australia several procedures for the determination of soil sulphur status were compared (Spencer and Freney, 1960) and the best correlations with plant dry matter yields were obtained using an extractant of potassium dihydrogen phosphate containing 500 $\mu g P/g$. The above extractant correlated with plant yields better than the "heat soluble sulphur" method, hot and cold water extractable sulphur, total sulphur, acetate extractable sulphur, HI-reducible sulphur and "reserve" sulphur.

It was concluded that this superior correlation was due to the ability of the phosphate to extract adsorbed sulphate. This implied that adsorbed sulphate was at least partly available, a new concept at that stage. Of almost equal precision was the micro-biological assay technique which involved the use of the fungal species Aspergillus niger. More recently another similar assay which used the algal species Nostoc sp. was found to be useful "as a guide to agricultural fertiliser recommendations" (Tchan et al., 1963). The algae were found to be able to utilise sulphate, cysteine and methionine. These microbiological assays seem theoretically ideal for evaluating available sulphur supplies as there is every likelihood that sulphur available to these micro-organisms will also be available to plants, even though our knowledge of soil sulphur forms will not be increased. However, recent work has either

overlooked this method or alternatively has disregarded it for practical reasons.

The findings of Fox et al., (1964) agreed with previous work (Spencer and Freney, 1960) which found that mono-calcium phosphate-extracted sulphur correlated best with sulphur "A" values. The calcium salt of phosphate was found to be preferable for practical reasons since no turbidity after filtering soil extracts was observed. This enabled rapid turbidometric methods to be employed and also increased the accuracy of the Johnson and Nishita method [errors are incurred when soil colloids are present (Freney, 1957)]. It was also found (Fox et al., 1964) that water extractable sulphur (mainly sulphate) provided a good measure of the sulphur status of some U.S.A. soils. However, Barrow (1961) believed that water soluble sulphur over-estimated amounts of available soil sulphur since sulphur was extracted by water from soil incubated with plant material, previously shown to immobilise available sulphur. As a result 0.15 percent calcium chloride solution was selected, which extracted less sulphur than the water. Calcium chloride was found to recover all sulphate added to soils except where a considerable sesquioxide content was present (Barrow, 1961). This neutral salt solution has been shown to correlate well with plant growth (Williams and Steinberg's, 1959) although it does not determine adsorbed sulphate (Ensminger and Freney, 1966).

Although water has proved to be a useful extractant for sulphur status assessment (Fox et al., 1964) many soils contain so little sulphate (which constituted practically all the water soluble sulphur) that poor correlations should be occasionally expected.

Various guidelines for indicating sulphur deficiency have been suggested, for instance Fox et al., (1964) proposed that where 0-6 µgS/g soil of calcium phosphate extractable soil sulphur was obtained, response to added sulphur was assured. Similarly, in the range 6-10 µgS/g sulphur a plant yield response was possible and when more than 10µgS/g was extracted a response was unlikely. However, these guidelines were proposed on the basis of pot experiments with alfalfa, a particularly deep rooting species which might respond to sulphur differently to other crops in the field.

Previously untried extracting reagents were investigated in the U.S.A. in conjunction with X-ray emission spectrography for measuring amounts of sulphur extracted (Roberts and Koehler, 1968). This method measured total sulphur in the extractant rather than sulphate-sulphur which is more commonly determined (after Freney, 1957). A curvilinear relationship, with good correlation, was obtained between total sulphur in wheat tops and sulphur extracted by 5 mM magnesium chloride. Molar lithium chloride was also tested and proven satisfactory in conjunction with the Johnson and Nishita method (1952).

Apparently the use of Morgans extractant still continues but, since the solution consists of N sodium acetate adjusted to pH 4.8, misleading results will be obtained. This is because at lower pH values increased adsorption of sulphate by soils has been noted (Ensminger, 1954).

Yet another extractant which has been long acclaimed is neutral ammonium acetate (McClung et al., 1959). This reagent extracts free sulphate-sulphur plus some organically combined sulphur. The reagent was found to be satisfactory by Spencer and Freney (1960) for some Australian soils, but has not proved popular amongst recent workers.

Bardsley and Lancaster (1960) have proposed the use of a fraction known as "reserve sulphur" to estimate sulphur status. This fraction is essentially the total organic sulphur (Cooper, 1971) and therefore over-estimates the sulphur taken up by plants in a growing season. Since good correlations were obtained with sulphur uptake by white clover the "reserve sulphur" may be a good measure of the long term supply of available sulphur.

Sulphur status prediction should be improved by measuring the soils capacity to mineralise organic sulphur to sulphate (in a similar manner to the methods used for nitrogen status assessment). Such an approach has been adopted by Harward et al., (1962) who achieved good correlation between extractable sulphur plus readily

mineralisable sulphur (measured by incubation methods) and percentage plant yield increase.

Recently, sulphur status prediction has been improved by taking into account other soil properties with the sulphur measurement. It was found (Barrow, 1969) that when the soils ability to adsorb sulphate was added to the multiple regression very good correlations were obtained with plant sulphur uptake. Similarly, Hoeft et al., (1973) attempted to improve correlations by including soil properties such as % organic matter, % sand, % subsoil sulphate-sulphur and pH. They found that only pH improved the prediction of yield response to added sulphur. This substantiates the previous work (Barrow, 1969) as it was well known that pH and sulphate adsorption capacity are very much inter-related (Ensminger, 1954).

Problems of using extractants with both gypsiferous and sesquioxide rich soils have been noted by several workers, e.g. Spencer and Freney (1960); Roberts and Koehler (1968). When phosphate solutions were used in conjunction with gypsiferous soils, under-estimates of available sulphur were obtained because phosphate depressed the solubility of calcium sulphate, much of which is available to plants by solution. However, these same phosphate solutions were most suited to measuring the sulphur status of sesquioxide rich soils (such as latosols) where most of the available sulphur was in the adsorbed form. However, although little direct evidence

is available indicating that adsorbed sulphate was plant available (but regressions are improved by addition of factors influencing adsorption (Barrow, 1969; Roberts and Koehler, 1968)). Very poor correlations were obtained when such soils were extracted with neutral salts incapable of displacing adsorbed sulphate (Roberts and Koehler, 1968).

Sulphur deficiency can also be diagnosed by plant analysis. This method would seem ideal as there are no dangers of measuring non-available sulphur fractions as is very likely with soil analysis. Also all soil properties (such as resistance to root growth or limited available water) are taken into consideration when sulphur taken up by plants is measured. However, the major problem of the method is that it assesses sulphur deficiency after it has actually occurred in the plants, by which time remedial action could be too late to eliminate yield reductions (Ensminger and Freney, 1966). Thus, soil analysis methods have the advantage in that they can predict the deficiency whereas plant analysis can only confirm the deficiency once it has occurred. It has been suggested that sulphur deficiency symptoms are seen in plants which have a N:S ratio greater than 20 or contain less than 0.2% sulphur in their dry weight (Cowling and Jones, 1970). Similar critical levels of sulphur have been reported in the literature for a wide range of crops (Freney et al., 1962).

To conclude, it seems that both soil analysis and plant analysis are useful, but are best used together to complement each other. This is because a fertiliser programme can only be planned in advance on the basis of soil analysis with the fertiliser effectiveness being monitored by plant analysis. Five main types of extractant have been proposed; those containing phosphate which will displace some adsorbed sulphate, those with a pH greater than 7 which will hydrolyse some organic sulphur, those containing acetate, neutral salt solutions and water. Based on literature reports to date, phosphate solutions show the most promise in accurately measuring sulphur status. Taking into consideration present knowledge the best measure of sulphur status must include both measures of adsorbed sulphate, and ready mineralisable sulphur, especially for most humid region soils, where much sulphur is made plant available by the mineralisation of soil organic sulphur.

3. MATERIALS AND METHODS

3.1 Soils

3.1.1 Soils of the Field Trial Sites

The two sites selected for sulphur balance studies in Berwickshire, S.E. Scotland (Blackaddermount Farm, NT 855534 and Dykegatehead Farm, NT 875517) were both on the Whitsome series soil. These soils are classed as brown forest soils and are loams of high natural fertility with imperfect drainage (Ragg, 1960). At both sites a rotational system of grass and cereals was practised.

Boghall Farm (NT 249654) was the site of similar sulphur balance studies in the Edinburgh area. The soil here was of the Easter Bush series which is an imperfectly drained brown earth derived from fluvio-glacial sands and gravels.

The sulphur response field trials in the West of Scotland were set up at Crichton Farm, Dumfries (NX 978738) and Woodhead Farm, Dumfries (NY 223679). The site at Crichton Farm was on Crichton series, a freely draining soil derived from Permian sandstones and conglomerates. The soils of Western Dumfriesshire have yet to be surveyed and no detailed information about the soils at Woodhead Farm was available. However the trial site was on imperfectly drained sandy clay loam with a history of a grass conservation/forage cereals, rotational practise.

3.1.2 Soils used in Pot Experiment I.

Soils from the two Berwickshire farms were used in this experiment. The soil was sampled from alongside the experimental plots from a depth of 0-25 cm , air dried and passed through a 2 mm sieve.

3.1.3 Soils used in Pot Experiment II

This pot experiment employed soils collected from the agricultural regions of south-east Scotland. Table 1 lists some basic soil properties of the nine soils, in addition to references where more detailed information on each soil can be obtained. All soils were collected from a depth of 0-20 cm , air dried, passed through a 2 mm sieve and stored in plastic buckets.

3.1.4 Soils used in general incubation studies.
(Sections 4.4.1-4.4.6)

Five soils were selected to provide a wide range of total sulphur levels, C:S ratios and organic carbon contents:- viz. Whitsome, Hobkirk, Hexpath, Linhope and an unnamed calcareous pelosol. Brief pedological descriptions and collection site details can be found in Table 2. All the soils were collected from a depth of 0-20 cm except for the Hexpath series which was sampled from 6-18 cm. This was to avoid the peaty top such that the mineral A horizon only, was sampled. All soils were air-dried, passed through a 2 mm sieve and stored in plastic buckets.

TABLE 1. Soils used in Pot Experiment II

Soil Series	Sampling Location	Parent Material	Textural Class of Topsoil	Drainage Class	Agricultural Use	Classification	References
Macmerry	NT 418710 nr. Macmerry, East Lothian	Mixed till	Sandy loam	Imperfect	Pasture	Brown earth	Ragg and Futty (1967)
Biel	NT 783715 nr. Cockburnspath, East Lothian.	Drifts derived from Carboniferous and Old Red Sandstone rocks	Clay loam	Imperfect	Continuous cultivation - cereals	Brown earth	Ragg and Futty (1967)
Eckford	NT 677476 nr. Greenlaw, Borders	fluvio-glacial or lacustro-glacial sands	sandy loam	Free	Permanent pasture	Brown forest soil	Ragg (1960)
Hobkirk	NT 729495 nr. Greenlaw, Borders.	Sandy loam till	Sandy loam	Free	Pasture/ cereals rotation	Brown forest soil	Ragg (1960)
Soutlope	NT 231638 nr. Edinburgh, Midlothian.	Andesitic lavas	loam	Free	Permanent pasture	Brown forest soil	Ragg (1960)
Humbie	NT 546707 nr. Haddington, East Lothian.	Mixed till	loam	Imperfect	Continuous cultivation	Brown forest soil	Ragg and Futty (1967)

TABLE 1. (Contd.)

Soil Series	Sampling Location	Parent Material	Textural Class of Topsoil	Drainage Class	Agricultural Use	Classification	References
Darvel	NT 243638 nr. Edinburgh, Midlothian.	Fluvio-glacial sands and gravels	sandy loam	Free	Continuous cultivation	Brown forest soil	Ragg and Futty (1967)
Pow	NO 667590 nr. Montrose, Tayside	Silty estuarine material	silty loam	Poor	Continuous cultivation	Non-calcareous gley.	
Boyndie	NO 695594 nr. Montrose, Tayside	Fluvio-glacial sands	sandy loam	Free	Market gardening and cereals	iron podsol	Laing (1976)

TABLE 2. Soils used in General Incubation Studies

Soil series	Sampling Location	Parent Material	Textural Class of Topsoil	Drainage Class	Agricultural Use	Classification	References
Whitsome	NT 875517 nr. Duns, Borders	Mixed till	loam	Imperfect	Cereals/ pasture rotation	Brown forest soils	Ragg (1960)
Hobkirk	NT 729495 nr. Greenlaw, Borders	Sandy loam till	sandy loam	Free	Cereals/ pasture rotation	Brown forest soil	Ragg (1960)
Hexpath	NT 672476 nr. Greenlaw, Borders	fluvio-glacial and lacustro-glacial sands	loamy sand	Free	Rough grazing	iron podsol	Ragg (1960)
Linhope	NT 625647 nr. Gifford, East Lothian	stony drift	loam	Free	Permanent pasture	Brown forest soil	Ragg and Futty (1967)
unnamed Calcareous pelosol	SX 785627 nr. Dartington, Devon	slaty head	clay	Imperfect	Permanent pasture	Brown earth	B.S.S.S. Handbook (1978)

3.1.5 Soils used in Incubation Experiment I (Section 4.4.7)

Forty soils were sampled from a depth of 0-20 cms, air dried, passed through a 2 mm sieve and stored in plastic buckets. All the soils described in Table 3 were used in addition to a selection of soils from Devon (Table 4) and other soils from various localities in Britain (Table 3).

3.1.6 Soils used in organic matter fractionation studies

The Kilmarnock, Stirling and Linhope soil series were used for this work. Soil samples, from 0-20 cms depth were air-dried and ground to pass a 2 mm sieve. The Kilmarnock and Stirling soils were collected from both continuously cultivated, and permanent pasture sites whilst the Linhope was collected from a rough grazing site. The Kilmarnock series was obtained from East Lothian (permanent pasture site NT 538769 and the continuously cultivated site NT 537768) and is a freely drained soil derived from tills of carboniferous sandstones and shales (Ragg and Futty, 1967). The Stirling series was collected from Tayside (permanent pasture site NO 147180 and the continuously cultivated site NO 154183) and is a poorly drained soil developed on estuarine clay silts (Laing, 1976). The Linhope series has been described in Table 2.

3.1.7 Soils used in the sulphur-35 studies

The Whitsome and Stirling (continuously cultivated site) soil series were used and are described in sections 3.1.1 and 3.1.6 respectively.

TABLE 3. Soils Used in Incubation Experiment I.

Soil series	Sampling location	Parent material	Textural Class of Topsoil	Drainage Class	Agricultural Use	Classification	References
Ragdale	TF 154675 Bardney, Lincolnshire	chalky boulder clay	clay	poor	Permanent pasture and arable samples used	Calcareous gley	B.S.S.S. Handbook (1978)
Denchworth	SP 085414 Worcestershire	clay shale	clay	poor	continuous cultivation	non-calcareous gley	
Wix	TM 131238 Tendring, Essex	collurium	sandy silt loam	poor	continuous cultivation	gley	B.S.S.S. Handbook (1977)
Kilmarnock	Permanent pasture NT 538769 continuous pasture NT 537768 Athelstaneford, East Lothian.	mixed till	clay loam	imperfect	continuous cultivation and permanent pasture samples used	brown forest soil	Ragg and Futty (1967)
Stirling	NO 154183 nr. Abernethy, Tayside	estuarine silty clays	silty clay	poor	continuous cultivation	non-calcareous gley	Laing (1976)

TABLE 3 (Contd.)

Soil series	Sampling location	Parent material	Textural Class of Topsoil	Drainage Class	Agricultural Use	Classification	Reference
Dreghorn	NT 483797 nr. Aberlady, East Lothian	gravelly ground	sandy loam	free	continuous cultivation	Brown forest soil	Ragg and Futty (1967)
Winton (sub-soil)	NT 458718 Pencaitland, East Lothian.	mixed till	clay loam	imperfect	ley pasture	Brown forest soil	Ragg and Futty (1967)
Cessford	NT 622388 nr. Earlston, Borders	loam till	sandy loam	poor	permanent pasture	non-calcareous gley	Ragg (1960)
Kedslie	NT 566381 nr. Earlston, Borders.	clay loam till	loam	imperfect	permanent pasture	brown forrest soil	Ragg (1960)

TABLE 4. Devon soils used in Incubation Experiment I

Soil series	Sampling location	Parent material	Textural Class of Topsoil	Drainage Class	Agricultural Use	Classification	References
Tedburn	SX 656984 nr. Okehampton, Devon.	shaly head	clay	poor	permanent pasture	non-calcareous gley	B.S.S.S. Handbook (1978)
Yellowland	SS 602169 Burnington Moor, Devon	sandstone, siltstone and shale	clay loam	imperfect	permanent pasture	non-calcareous gley	"
Dunsford	SX 654967 nr. Okehampton, Devon	shaly head	silty clay loam	imperfect	permanent pasture	Brown earth	"
Neath	SS 610160 Burrington, Devon	fine sandstone, siltstone and shale	silty clay loam	imperfect	permanent and ploughed pasture soils used	Brown earth	"
Halstow	SX 658983 nr Okehampton, Devon	shaly head	silty clay loam	imperfect	ley pasture	non-calcareous gley	"
Highweek	SX 766679 nr. Ashburton, Devon	slaty head	clay loam	imperfect	Barley	Brown earth	"

TABLE 4 (contd.)

Soil series	Sampling location	Parent material	Textural Class of Topsoil	Drainage Class	Agricultural Use	Classification	References
Trusham	SX 777619 Dartington, Devon	doleritic head	clay loam	imperfect	ley pasture	Brown earth	B.S.S.S. Handbook (1978)
Holsworthy	SS 610164 Burrington, Devon	fine sandstone, siltstone and shale	silty clay loam	imperfect	permanent pasture	Brown earth	"
Moorgate	SX 730855 Dartmoor, Devon	head containing granite	loamy sand	free	permanent pasture	Podsol	"
unnamed non-calcareous pelosol	SX 790630 Dartington, Devon	clay over limestone	clay loam	imperfect	ley pasture	Brown earth	"

3.2 Analytical techniques

3.2.1 Sulphate-sulphur

The automated turbidimetric method of Sinclair (1973) was used to determine sulphate-sulphur. The sulphate in the sample is precipitated as barium sulphate under controlled pH conditions. This precipitate is kept in suspension by using surfactants, measured by optical adsorption and compared with standards prepared from analytical reagent grade sodium sulphate.

3.2.2 Total sulphur in soil and plant material

3.2.2.1 Chemical oxidation technique

The chemical oxidation method of Steinbergs *et al.*, (1962) was used to determine total sulphur. The soil or plant material was mixed with sodium bicarbonate and silver oxide and ashed at 550°C in a muffle furnace overnight. Instead of employing the Johnson and Nishita (1952) finish an automatic turbidimetric method was used (Sinclair, 1973) as follows. The oxidised material, contained in a 5 ml porcelain crucible, was placed in a polythene 100 ml centrifuge tube. This was then extracted with 20 ml of monocalcium phosphate (made up to a concentration of 500 µg phosphorus/g) containing 446 ml of molar hydrochloric acid. This quantity of acid is equivalent to the 0.25 g of sodium bicarbonate used in the oxidation. After extraction, which entailed orbital shaking for one hour, the solution was filtered through a Whatman No. 42 filter paper. The filtrate was then

analysed for sulphate using the Sinclair (1973) automated turbidimetric method. This method was chosen when only small samples of herbage could be obtained. The method was also used for soils and freeze dried organic matter extracts. In the last case the sodium bicarbonate was applied in solution and then evaporated to dryness to ensure adequate mixing with the sample.

3.2.2.2 X-Ray Fluorescence Spectrometry

The x-ray fluorescence method of Evans (1970) was used to determine total sulphur in herbage samples collected from the field trial sites. The plant tissue (1g) was ball-milled with powdered cellulose (1g) and then pelleted using a pressure of 5 tons/square inch. Some soil total sulphur determinations were also made using this method together with the sample preparation techniques of Norrish and Hutton (1964). This entailed pelleting a 1g sample of ball-milled soil with a boric acid backing. The spectrograph, a Philips PW 1540 all vacuum model, was used in conjunction with a Philips PW 1010 x-ray generator, a pentaerythritol crystal and a gas flow proportional counter. Standards were prepared by additions of known amounts of analytical-reagent grade ammonium sulphate to a ground, bulk herbage (or soil, where applicable) sample.

3.2.3 Hydriodic acid-reducible sulphur in soil

The HI-reducible sulphur content of soils and organic matter extracts was determined using the reduction mixture of Freney et al., (1969). This mixture contained

hydriodic acid (specific gravity 1.7), formic acid (90%), and hypophosphorous acid (50%) in the ratio of 4:1:1 by volume. The resultant, liberated hydrogen sulphide was collected in molar sodium hydroxide solution and the sulphide content determined by titration with mercuric acetate using dithizone as indicator (Archer, 1956). Approximately 50 mg of soil or an amount of organic matter extract containing between 5 µgS and 20 µgS, was used for each determination. When liquids were analysed evaporation of the sample to dryness within the reaction vessel was carried out to prevent dilution of the reduction mixture.

3.2.4 Carbon-bonded sulphur in soil

The carbon-bonded sulphur in soil was determined by calculating the difference between total soil sulphur and HI-reducible soil sulphur since direct methods of determination are considered unreliable (Freney et al., 1970).

3.2.5 Sulphate in rainwater and hydrogen peroxide solutions

Where samples free from interfering anions were collected such as rainwater and hydrogen peroxide (from the SO_2 monitoring device) samples the Thorin method (Persson, 1966) of analysing for sulphate was chosen in preference to the less sensitive automated turbidimetric method of Sinclair (1973). This automated colorimetric method involves the measurement of barium ions remaining after precipitation of the sulphate in the sample with a barium

perchlorate solution. The excess barium was indicated with Thorin. When rainwater samples were analysed the sample was first passed through a column of Dowex 50W-X8 cation exchange resin (Bio-Rad Laboratories, California) to remove interfering cations. The diluted hydrogen peroxide samples needed no pretreatment. Standards were prepared from analytical-reagent sulphuric acid and standardised with analytical-reagent grade anhydrous sodium carbonate.

3.2.6 Extractable Soil Sulphate

Soil (20g) was extracted with 50 ml of a mono-calcium phosphate solution containing 500 µgP/g (Ensminger, 1954). The mixture was shaken for 30 minutes on an orbital shaker then filtered through a Whatman No. 42 filter paper. The filtrate (20 ml) was similarly shaken for 30 minutes with purified charcoal (0.2g) and 5 ml of an acid mixture containing acetic acid, orthophosphoric acid and hydrochloric acid (Sinclair 1973). The mixture was then filtered through a Whatman No. 42 filter paper and the sulphate content determined by the automated turbidimetric technique.

3.2.7 Extractable plant sulphate

Dried, milled plant material (0.5g) was extracted with 50 ml of cold distilled water by orbital shaking for 30 minutes. The extract was filtered through a Whatman No. 42 filter paper. The filtrate was then treated with charcoal and acid mixture, as described in 3.2.5 above, to

remove interfering coloured organic molecules. The sulphate content of the decolorised solution was determined by the automated turbidimetric method (Sinclair, 1973).

3.2.8 Extractable plant nitrate

An automated method (Henriksen and Selmer-Olsen, 1970) was selected to measure nitrate levels in herbage samples. Approximately 0.5g of herbage was shaken with 50 ml of cold distilled water for 30 minutes, filtered using a Whatman No. 42 filter paper and the appropriate dilutions then made. Fresh extracts are then fed into the auto-analyser system where the nitrate is first reduced to nitrate which then undergoes diazotisation with sulphanilamide and coupling with N-1-napthylethylene-diamine to form a highly coloured dye. Standards were prepared from analytical-reagent grade potassium nitrate. Sample dilutions were always large enough to ignore the effect of coloured organic components in the extracts.

3.2.9 Total Nitrogen in soil and plant material

Total nitrogen was determined, on soil and herbage samples, by the semimicro-Kjeldahl method described by Black (1965). Additions of potassium sulphate and a copper catalyst were made to all samples to speed up the digestion stage. In the case of herbage samples hydrogen peroxide was added to the digestion mixture to further speed up the digestion process. The Kjeldahl method was investigated to assess whether the value obtained for total

nitrogen included nitrate-nitrogen. This was examined by making known additions of potassium nitrate to the herbage before digestion. Also amounts of pure potassium nitrate were digested in the absence of herbage. It was found that no added nitrate was recovered in the presence or absence of herbage. On addition of the hydrogen peroxide, fumes of nitrogen dioxide were observed indicating oxidation of the nitrate. Therefore the "total nitrogen" value obtained by the Kjeldahl method does not include nitrate-nitrogen. Ammonium-nitrogen was determined in the digest by steam distillation and titration with standard acid (Black, 1965).

3.2.10 Soil Organic Matter

Organic carbon content of soils was determined by the method of Tinsley (1950) as modified by Bremner and Jenkinson (1960). This method involves a wet oxidation step followed by titrimetric measurement of unreacted potassium dichromate. Organic matter was determined by multiplying per cent carbon by 1.74.

3.2.11 Ash Content of Organic Matter Extracts

The amount of mineral matter in freeze-dried organic matter fractions was determined by combustion in a muffle furnace maintained at 450°C. overnight.

3.2.12 Soil pH

The pH of soil samples was determined in a 1:2.5 ratio of soil to distilled water. The soil/water suspension was allowed to equilibrate for two hours. A pH meter

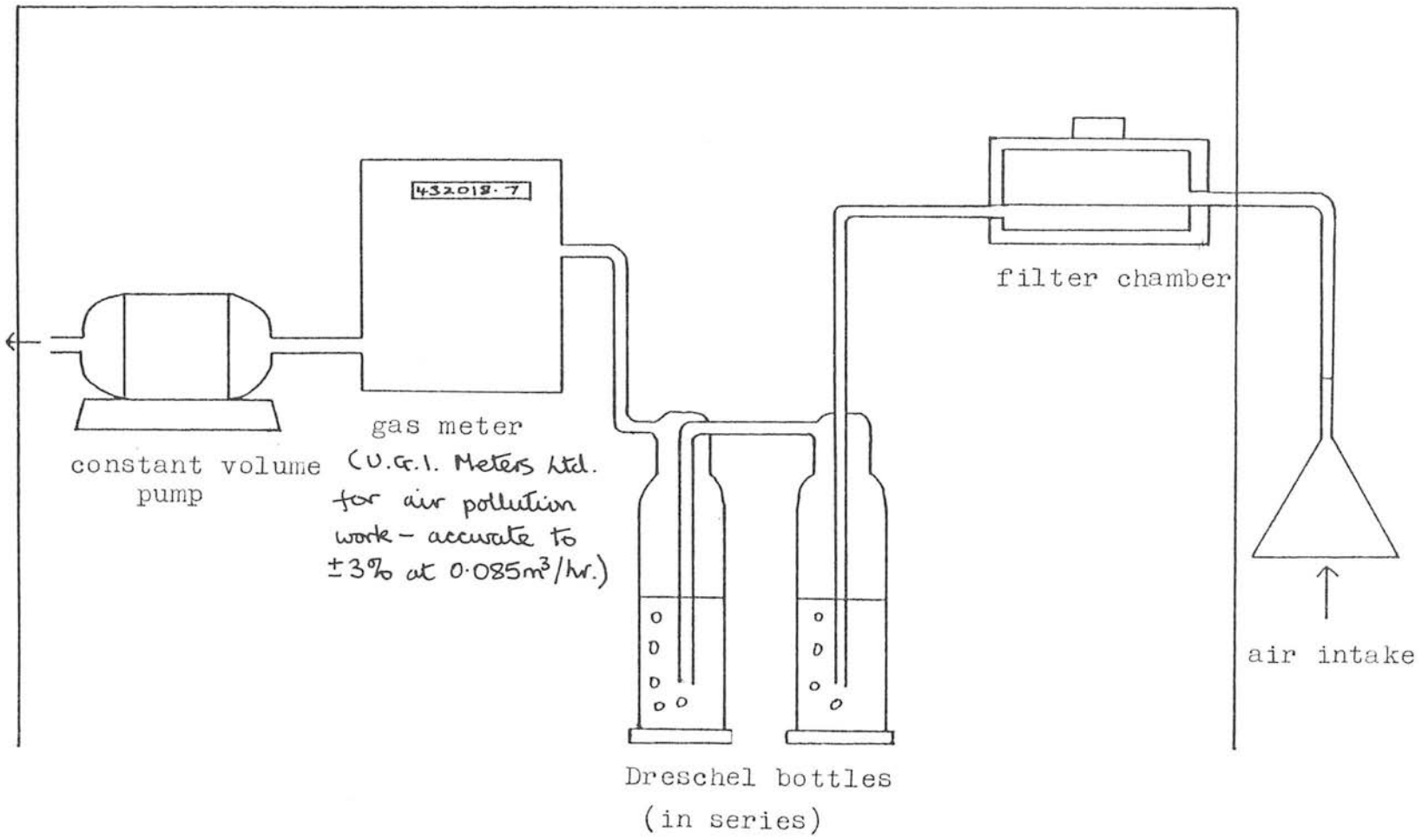
(Electronic Instruments Ltd., Model No. 7020) was used in conjunction with glass and reference electrodes calibrated at pH 4 and pH 7 with buffer solutions.

3.2.13 Determination of the Atmospheric Inputs of Sulphur including Dry Deposition and Particulate Deposition

Reference to Fig. 1 shows the air sampling apparatus employed. The set-up is similar to "the daily instrument" described in the National Survey of Smoke and Sulphur Dioxide Instruction Manual (1966). However the Dreschel bottles containing the 1 volume hydrogen peroxide were changed and analysed weekly. Two dreschel bottles were arranged in series to check for complete sulphur dioxide absorption. Atmospheric concentrations of sulphur dioxide were obtained from knowledge of the volume of air pumped through the apparatus and the amount of sulphate absorbed. Particulate sulphate was measured weekly by extracting the filter paper in monocalcium phosphate solution containing 500 µg phosphate/g.

The filter paper was shaken for one hour with the monocalcium phosphate solution (20 mls), filtered through a Whatman No. 42 filter paper and the filtrate analysed for sulphate by the turbidimetric technique of Sinclair (1973). This procedure was chosen in preference to a total sulphur measurement of the particulate matter since it would be more meaningful in terms of crop available sulphur. The sulphate in the hydrogen peroxide solution was determined by the Thorin technique (Persson, 1966). Atmospheric sulphur dioxide concentrations were converted into amounts

Figure 1. The apparatus used to determine atmospheric sulphur



of sulphur deposited in the field by use of a deposition velocity of 0.8 cm sec.$^{-1}$ calculated experimentally by Fowler (1978). The same worker similarly determined a deposition velocity of 0.06 cm sec.$^{-1}$ for the particulate matter.

3.2.14 Determination of rainfall sulphur

Rainfall was collected using a glass funnel mounted 25 cms above ground level. The rainwater filtered through a plug of glass wool placed in the neck of the funnel. The plastic collection vessel was changed monthly and samples were then analysed for sulphate using the Thorin method of Persson (1966).

3.3 Field Experiments

3.3.1 Berwickshire Field Experiments

In 1976 sites at Dykegatehead Farm and Blackadder-Mount Farm, both in Berwickshire, were sown with Italian Ryegrass (R.V.P.). This sowing date ensured a good sward establishment such that trials would begin in Spring 1977 and be continued over two growing seasons. Eight plots were marked out at both sites, each measuring 8m x 2m. Each plot was then assigned one of the following sulphur treatments, top dressed as calcium sulphate; 0 kgS/ha (two plots), 5, 10, 20, 40, 80 and 160 kgS/ha. For the second year (1978) the allocation of treatments was modified as follows: 0, 40, 80 and 160 kgS/ha with a replication of two per treatment. During 1977 each plot initially received 120 kgN/ha applied as a commercial grass

fertiliser. Cuts were made throughout the growing season to coincide with cuts made at the farm for silage and hay, and yields were recorded. After each of the first two cuts 100 kgN/ha was added to all plots and 60 kgN/ha after the third cut. During 1978 a similar nitrogen application programme was used except that "Nitram" (I.C.I.) and laboratory reagents were employed since it was discovered that the compound fertiliser previously used contained a small amount of sulphur.

Samples of grass were collected at each harvest from all plots, dried overnight at 60°C, and milled to pass a 1 mm sieve. Soil samples were taken periodically throughout the experiment to monitor sulphate levels in the topsoil. The soils were sampled from 0-20 cm using a screw auger. Rainfall was continuously sampled at both sites and analysed monthly for sulphate. The atmospheric sulphur dioxide concentration was monitored continuously from April 1978 to April 1979 at Dykegatehead.

3.3.2 West of Scotland Field Experiments

Permission was obtained to set up sulphur response experiments at Woodhead Farm and Crichton Farm (both in Dumfriesshire) during early April 1979. Eight plots of 8m x 2m were marked out at each site on established ryegrass swards. Two sulphur treatments of 0 and 50 kgS/ha were applied in quadruplicate, as top dressed calcium sulphate, at both sites. In addition all plots received 120 kgN/ha as "Nitram" (I.C.I.) and a further 100 kgN/ha, also as "Nitram", after the first harvest. Two cuts were made to

coincide with silage cuts made by the farmer. Dry-matter yields were recorded. Herbage samples from each plot were taken for analysis. Soil samples were collected from 0-20 cms using a screw auger before sulphur fertilisation and after each harvest.

3.3.3 Edinburgh Area Field Experiments

An area of established perennial ryegrass (R.V.P. Hay Pasture) sown in 1976, was obtained at Boghall Farm near Edinburgh. During the beginning of April 1979 twenty-two plots measuring 5m x 1.8m were marked out and treated with calcium sulphate to give the following sulphur applications; 0, 10, 20, 40, 60, and 100 kgS/ha. The zero treatment was applied to four plots and the remaining treatments were each applied to three plots. Treatments were similarly applied in April 1980 except that one zero and one 100 kgS/ha plot remained unfertilised as they were required for leaching studies. The plots also received 120 kgN/ha as "Nitram" (I.C.I.) and a further 100 kgN/ha after the first cut. A particularly dry season enabled only two harvests to be made. Dry weight yields were recorded and samples from each plot were collected for analysis. Soil samples were taken before treatment application and at times when soil moisture conditions allowed sampling at 0-20 cms with a screw auger. Rainfall was collected continuously over the experimental period and analyed for sulphate monthly. The atmosphere was sampled continuously from May 1979 until autumn 1980, as described in 3.2.1.3 above.

3.4 Pot Experiments

3.4.1 Experiment I. The sulphur status of Whitsome series soil.

Whitsome series soil from Blackadder mount and Dykegatehead was collected and prepared as described in 3.1.2. Soil (1.5kg) was placed into 6" pots and sown with 0.4g of Perennial Ryegrass (523) during mid June 1978. A factorial design was employed with two nitrogen treatments, two soils and three sulphur treatments of 0, 8 and 40 µgS/g soil which is equivalent to 0, 20 and 100 kgS/ha (assuming 2,500 tonnes topsoil per hectare). Each treatment was prepared in triplicate giving 2 x 2 x 3 x 3 = 36 pots. The sulphur was applied as a solution of sodium sulphate to the soil surface prior to sowing. The high nitrogen treatment consisted of applying 48 µgN/g soil (120 kgN/ha) soon after sowing and then 40 µgN/g soil (100 kgN/ha) after each harvest. The low nitrogen pots only received 40 µgN/g soil (100 kgN/ha) midway through the growing season. The nitrogen was added as ammonium nitrate in solution. Both soils had been previously analysed for potassium and phosphorus and were found to be adequately supplied with both nutrients. Pots were maintained at field capacity by regular watering with deionised water. By mid October 1978 heating and illumination was used to maintain glasshouse conditions of a 16 hour photoperiod and constant temperature of 20°C. The pots were arranged randomly in the glasshouse.

At each harvest the grass was cut to within 1 cm of the soil surface, dried overnight at 60°C and the dry matter yields recorded. All herbage was milled and retained for analysis. Three cuts were made on the high nitrogen treatments. Only two cuts were made on the low nitrogen treatments due to insufficient growth. At the final harvest the soil in each pot was sampled using a 1 cm boring tube, air dried and analysed for extractable sulphate.

3.4.2 Experiment II. The sulphur status of nine Scottish Soils.

Nine soils were collected and prepared as described in section 3.1.2. Soil (1.5kg) was placed into six inch pots and sown with 0.4g of perennial ryegrass (S23) during late July, 1979. A factorial design was used combining nine different soil series, two sulphur treatments of 0 and 40 μgS/g soil, (100 kgS/ha) each in duplicate, giving 9 x 2 x 2 = 36 pots. The sulphur was applied as sodium sulphate in solution to the soil surface prior to sow ing. All pots were additionally given 48 μgN/g soil (120 kgN/ha) at the time of sowing and a further 40 μgN/g soil (100 kgN/ha) after each harvest. The nitrogen was added as ammonium nitrate in solution. At sowing all pots were fertilised with 10 μgK/g soil (25 kgK/ha) (applied as potassium chloride in solution) and 8 μgP/g soil (20 kgP/ha) (applied as sodium dihydrogen orthophosphate in solution). Pots were maintained at field capacity by watering with deionised water to a predetermined weight.

When climate began to limit growth (mid October) the glasshouse was heated to 20°C and illumination provided a 16 hour photoperiod. The pots were arranged randomly in the glasshouse.

Three harvests were made during the experiment (July 1979 → Jan 1980) when the grass was cut to within 1 cm of the soil surface. Dry matter yields per pot were determined and the whole sample milled and retained for analysis. At the final harvest the soil was removed from each pot, air dried, separated from the roots and ground to pass a 2 mm sieve. The soil was then analysed for extractable sulphate.

3.5 Incubation Studies

The method of Swift (1977) was adopted, for all the incubation studies, with only slight modification. Soils (20g) were intimately mixed with a purified acid-washed sand (60g) and 15 ml of water inside a wide necked 250 ml conical flask. It was found necessary to further purify the acid washed sand (May and Baker Ltd.) by boiling with 20 volumes hydrogen peroxide followed by boiling with concentrated hydrochloric acid. The flasks were loosely stoppered with cotton wool bungs to ensure adequate aeriation. The weights of the flasks were checked every two days and distilled water added to replace that lost by evaporation. The flasks were incubated in darkness at the appropriate temperature and for the desired length of time. Control flasks were set up, containing sand and water, and incubated alongside the soils. Treatments were

incubated in duplicate throughout. Values of net mineralised sulphate were obtained by determining the extractable sulphate level in the soil both before and after a period of incubation.

3.6 Organic matter fractionation studies

The methods employed in this section are considered under 4.6 as methods were constantly being developed and require simultaneous discussion with the results.

3.7 Studies involving the use of Sulphur-35.

Sulphur-35 was supplied as carrier free inorganic sulphate in aqueous solution, pH 6-8, by The Radiochemical Centre, Amersham. Since this isotope is a soft beta particle emitter (energy maximum .167 MeV), liquid scintillation was chosen as the assay technique. The sample, in solution, was mixed with 5 mls of micellor scintillator NE 260 (Nuclear Enterprises Ltd.) in a small glass vial and counted using a Panax Reigate Series counter. Where coloured samples were encountered quenching was compensated for by internal standardisation. Standards were prepared in the same solutions in which samples were counted to allow for any chemical quenching.

3.7.1 Incubation of Whitsome series soil with sulphur-35.

One kilogram of Whitsome series soil (air dried and ground to pass a 2 mm sieve) from Dykegatehead Farm (see 3.1.1 for description) was mixed with 2 mCi of sulphur-35 (as sulphate).

Thorough isotope/soil mixing was ensured by sprinkling the isotope in solution onto aliquots of the soil and then drying the mixture under an infra-red lamp. The aliquots were then mixed in with the bulk of the soil by overnight end over end shaking. In addition to the carrier free isotope, 15 mg of sulphur as sodium sulphate in solution was also added. The soil was then split into two 500 g portions with one portion receiving 0.5% glucose. Each portion was then placed into a large crystallising dish, watered to three-quarters field capacity, and covered with a perforated foil lid. The soils were incubated in darkness at 20°C. for 75 days with regular maintenance of the desired water status and occasional mixing of the soil. On completion of the incubation period both portions of soil were air dried and ground to pass a 2 mm. sieve.

Each portion was analysed for extractable sulphate, total sulphur and HI-reducible sulphur. Additionally the specific activity of each of the above forms of sulphur was determined. The extractable sulphate was determined using the whole soil whilst the other sulphur forms were determined on leached soil samples. The leaching, using 0.01M calcium chloride, was performed until no activity could be detected in the leachate. The activity of the total sulphur was measured by counting the extracted combustion mixture obtained from the chemical oxidation technique for determining total sulphur. The activity of the HI-reducible sulphur was measured by treating 0.75g

of soil with the reduction mixture and collecting the labelled sulphide in 5 ml of molar sodium hydroxide. This was then made up to 25 ml in a volumetric flask and counted.

3.7.1.1 Re-incubation of labelled Whitsome series soil.

The labelled soil prepared by incubation with isotope in 3.7.1 above was re-incubated as described in section 3.5, entitled Incubation Studies. The labelled soil was, however, first leached with 0.01M calcium chloride until no activity could be detected in the leachate. This was performed to remove excess sulphate-35. The soil in the leaching tubes was allowed to dry for two days to allow removal of the soil without causing structural breakdown. The soil was then air dried. To replace the nutrients leached out by this procedure 40 μgN/g soil, 20 μgP/g soil and 20 μgK/g soil were added to each conical flask as ammonium nitrate, sodium dihydrogen orthophosphate and potassium chloride respectively. Also a starter innoculum, prepared from previously wetted Whitesome series soil, was added to each flask. The soils, originally incubated with two levels of glucose, were re-incubated at 30°C in darkness for 0, 7, 14, 21, 28 and 50 days so that rates of sulphate-32 and sulphate-35 mineralisation could be monitored.

3.7.2 Incubation of Stirling series soil with sulphur-35.

Soil of the Stirling series (1 kg), described in section 3.1.6. , was air dried and ground to pass a 2 mm sieve. The soil was mixed with 5 mCi of sulphate-35, as

described in the previous section, and 5 mg of sulphate-32. The soil was again divided into two 500 g portions and incubated and watered as in 3.7.1 except that both portions received glucose (0.5 percent). The incubating soil was sub-sampled after 10, 25, 50 and 75 days. At each sampling time approximately 50 g of soil was immediately air dried, ground to pass a 2 mm sieve and stored at 4°C.

3.7.2.1 Re-incubation of labelled Stirling series soil

The labelled Stirling soil was re-incubated using the same procedure developed for the Whitsome soil and described in 3.7.1.1 above. However in this instance, portions of soil were quickly leached using a Buchner funnel and Whatman No. 42 filter paper. The leached soil pad was then immediately air dried.

4. RESULTS AND DISCUSSION

4.2 Field Experiments

The introductory section showed in detail how a combination of increased yields, introduction of Clean Air Acts and widespread use of sulphur-free fertilisers was likely to cause marginal sulphur sufficiency in areas of the U.K. receiving low atmospheric inputs of sulphur. This section investigates the sulphur balance in areas of low and average atmospheric sulphur input. The investigation was carried out by monitoring inputs to and losses of, sulphur from the soil/plant system. In addition the effects of added sulphur on plant yield and plant chemical composition and on soil sulphur status are examined. The measurement of sulphur inputs and losses also enabled an estimation of sulphur mineralisation over a growing season to be made and hence the significance of soil sulphur can be assessed.

4.2.1 Berwickshire Field Trials

The trials in Berwickshire were set up to assess the response of ryegrass to added sulphur; investigate the effect of added sulphur on the mineral nutrition of ryegrass and to construct a sulphur balance sheet for the ryegrass crop. Construction of a balance sheet would enable quantification of the sulphur contribution made by the mineralisation of sulphur in the soil over the growing season. Experimental design and crop husbandry details can be found in section 3.3.1.

4.2.1.1 Sulphur levels in herbage

Data (Tables 5, 6, 7 and 8) clearly shows that for most cuts, added sulphur increased the total sulphur content of the herbage at both sites and for both years. However even the control plots produced herbage within the critical region for both plant and animal nutrition which is 0.15-0.16% sulphur. The herbage from Blackaddermount contained more sulphur than that from Dykegatehead during early 1977 but no difference between sites was evident at the end of the 1977 season nor during the whole of 1978. Overall the sulphur content of the herbage during 1977 was higher than that of 1978 probably because the commercial compound fertiliser used in 1977 was replaced with sulphur-free laboratory reagents in 1978. Climatic differences between the two seasons affected yields and the chemical composition of the herbage. It is also noticeable that the sulphur content of the herbage significantly increased throughout the growing season. This phenomenon, also noted by other workers (Murphy, 1978), is probably a plant physiological effect rather than a soils effect.

The sulphate levels in the herbage did not show such distinct trends as the total sulphur levels (Tables 9, 10, 11 and 12). This is not suprising since sulphate levels will be more transient and will indicate the balance between plant uptake of sulphate and its subsequent incorporation into organic sulphur. Both of these plant functions will vary greatly with climate and plant age.

TABLE 5. The total sulphur content of herbage harvested from Blackaddermount during 1977.

Added S (kgS/ha)	Total S* (gS/100g D.M.)				
	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5
0	0.21	0.27	0.31	0.34	0.41
5	0.21	0.27	0.34	0.35	0.40
10	0.22	0.23	0.34	0.34	0.37
20	0.22	0.28	0.33	0.34	0.37
40	0.23	0.34	0.37	0.34	0.37
80	0.27	0.34	0.39	0.37	0.47
160	0.24	0.35	0.41	0.39	0.42

Standard Error of cut mean ± 0.008. Standard Error of Treatment mean ± 0.009. Effect of S and cut both significant ($P < 0.01$).

*mean of duplicate determinations.

TABLE 6. The total sulphur content of herbage harvested from Dykegatehead during 1977.

Added S (kgS/ha)	Total S* (gS/100g D.M.)			
	Cut 1	Cut 2	Cut 3	Cut 4
0	0.15	0.20	0.21	0.43
5	0.17	0.23	0.34	0.40
10	0.17	0.21	0.34	0.37
20	0.20	0.22	0.33	0.37
40	0.23	0.23	0.37	0.37
80	0.20	0.29	0.39	0.47
160	0.27	0.35	0.41	0.42

Standard Error of cut mean ± 0.013. Standard Error of Treatment mean ± 0.017. Effect of S significant ($P < 0.01$) Effect of cut significant ($P < 0.001$).

*mean of duplicate determinations.

TABLE 7. Total sulphur content of herbage harvested from Blackaddermount during 1978.

Added S (kgS/ha)	Total S* (gS/100g D.M.)		
	Cut 1	Cut 2	Cut 3
0	0.20	0.19	0.22
40	0.27	0.24	0.25
80	0.32	0.23	0.31
160	0.28	0.24	0.29
Standard Error	± 0.02	± 0.01	± 0.01

Effect of added S significant at cut 1 and cut 3 ($P < 0.05$) and not significant at Cut 2.

*mean of two replicate plots.

TABLE 8. Total sulphur content of herbage harvested from Dykegatehead during 1978

Added S (kgS/ha)	Total S* (gS/100g D.M.)		
	Cut 1	Cut 2	Cut 3
0	0.19	0.19	0.20
40	0.22	0.22	0.20
80	0.22	0.28	0.31
160	0.30	0.26	0.29
Standard Error	± 0.02	± 0.02	± 0.02

Effect of added S significant at cut 3 only ($P < 0.05$)

*Mean of two replicate plots.

TABLE 9. Sulphate-sulphur content of herbage harvested from Blackaddermount during 1977.

Added S(kgS/ha)	Sulphate-sulphur* (gS/100g D.M.)				
	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5
0	0.11	0.15	0.16	0.13	0.18
5	0.10	0.15	0.18	0.10	0.10
10	0.11	0.13	0.18	0.11	0.15
20	0.12	0.15	0.19	0.11	0.14
40	0.12	0.21	0.20	0.09	0.15
80	0.13	0.23	0.24	0.17	0.22
160	0.15	0.23	0.26	0.16	0.20

Standard error of cut mean ± 0.0074. Standard error of treatment mean ± 0.0088. Effect of S and cut both significant ($P < 0.001$)

TABLE 10. Sulphate-sulphur content of herbage harvested from Dykegatehead during 1977

Added S (kgS/ha)	Sulphate-sulphur* (gS/100g D.M.)			
	Cut 1	Cut 2	Cut 3	Cut 4
0	0.07	0.09	0.08	0.09
5	0.07	0.12	0.09	0.10
10	0.07	0.11	0.10	0.11
20	0.10	0.11	0.09	0.11
40	0.12	0.12	0.12	0.12
80	0.12	0.17	0.14	0.17
160	0.17	0.25	0.15	0.18

Standard error of cut mean ± 0.0063. Standard error of treatment mean ± 0.0083. Effect of S significant ($P < 0.001$) Effect of cut significant ($P < 0.01$)

*mean of duplicate determinations.

TABLE 11. Sulphate-sulphur content of herbage harvested from Blackaddermount during 1978.

Added S (kgS/ha)	Sulphate-sulphur* (gS/100g D.M.)		
	Cut 1	Cut 2	Cut 3
0	0.08	0.07	0.07
40	0.15	0.10	0.13
80	0.19	0.12	0.19
160	0.17	0.12	0.15
Standard error	± 0.0156	± 0.0104	± 0.0140

Effect of added S significant at cut 1 and cut 3 ($P < 0.05$), effect of added S not significant at cut 2.

TABLE 12. Sulphate-sulphur content of herbage harvested from Dykegatehead during 1978.

Added S (kgS/ha)	Sulphate-sulphur* (gS/100g D.M.)		
	Cut 1	Cut 2	Cut 3
0	0.04	0.06	0.07
40	0.07	0.09	0.10
80	0.15	0.16	0.15
160	0.22	0.12	0.15
Standard error	± 0.0039	± 0.0118	± 0.0217

Effect of added S significant at cut 1 ($P < 0.001$), significant at cut 2 ($P < 0.05$) and not significant at cut 3.

*mean of two replicate plots.

During 1977 both sites showed increased sulphate levels up to mid season, a drop at the penultimate cut and then a slight increase at the final harvest. A similar trend was observed at Blackaddermount for 1978 but at Dykegatehead a gradual increase in plant sulphate over the whole season was seen.

Recent work (Freney et al., 1978) has proposed using the percentage of sulphur occurring as sulphate in the plant as a diagnosis of sulphur deficiency. These workers reported critical values of 10 percent for a wheat crop, that is where at least 10 percent of the sulphur occurs as sulphate then a response to added sulphur was unlikely. When the percentage of the herbage sulphur occurring as sulphate is calculated for the field trial data (Tables 13 and 14) it can be seen that none of the values approached the critical value mentioned for wheat plants. Larger additions of sulphur (80 and 160 kgS/ha) greatly increased the proportion of sulphur occurring as sulphate which indicated luxury uptake.

4.2.1.2 Nitrogen levels in herbage.

Sulphur fertilisation has been found to increase crude protein-N production in the ryegrass crop (McLaren et al., 1978). Therefore both crude protein-N and nitrate levels were monitored in the herbage throughout the experiment to investigate any such similar nutritional quality improvements. Crude protein-N levels (Tables 15 and 16) were not increased by sulphur

TABLE 13. The percentage of the total herbage sulphur occurring as sulphate - 1977 trials

(a) Blackaddermount

Added Sulphur (kgS/ha)	% of total sulphur as sulphate				
	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5
0	53	56	52	38	44
5	49	57	54	30	25
10	49	56	54	33	41
20	55	54	58	32	38
40	52	62	55	27	41
80	48	69	62	46	47
160	62	67	64	42	47

(b) Dykegatehead

Added Sulphur (kgS/ha)	% of total sulphur as sulphate			
	Cut 1	Cut 2	Cut 3	Cut 5
0	46	44	38	21
5	42	53	27	25
10	42	53	30	30
20	51	50	27	29
40	52	52	33	32
80	59	59	36	36
160	63	72	37	43

TABLE 14. The percentage of the total herbage sulphur occurring as sulphate - 1978 trials.

(a) Blackaddermount

Added sulphur (kgS/ha)	% of total sulphur as sulphate		
	Cut 1	Cut 2	Cut 3
0	41	36	33
40	57	43	51
80	60	50	62
160	61	51	51

(b) Dykegatehead

Added sulphur (kgS/ha)	% of total sulphur as sulphate		
	Cut 1	Cut 2	Cut 3
0	20	31	33
40	34	43	51
80	67	56	49
160	73	47	52

TABLE 15. Kjeldahl-nitrogen content of herbage harvested during 1977

(a) Blackaddermount

Added S(kgS/ha)	Nitrogen (gN/100g D.M.)				
	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5
0	1.56	1.83	2.82	3.76	3.89
5	1.75	2.02	2.96	3.89	4.33
10	1.69	1.97	2.92	4.18	4.29
20	1.64	2.07	2.83	4.06	4.30
40	1.65	2.04	2.59	4.10	4.17
80	1.47	1.88	2.77	3.50	4.08
160	1.47	1.77	2.48	3.84	3.95

Standard error of cut mean ± 0.05. Standard error of treatment mean ± 0.05. Effect of S and cut both significant ($P < 0.001$)

(b) Dykegatehead

Added S(kgS/ha)	Nitrogen (gN/100g D.M.)			
	Cut 1	Cut 2	Cut 3	Cut 4
0	1.38	1.79	2.02	3.48
5	1.54	1.97	1.89	3.71
10	1.41	1.85	1.96	3.49
20	1.48	1.70	2.05	3.84
40	1.49	1.85	1.98	3.49
80	1.26	1.83	1.73	3.36
160	1.69	2.16	2.00	4.03

Standard error of cut mean ± 0.04. Standard error of treatment mean ± 0.006. Effect of S significant ($P < 0.01$) Effect of cut significant ($P < 0.001$).

TABLE 16. Kjeldahl-nitrogen content of herbage harvested during 1978

(a) Blackaddermount

Added S (kgS/ha)	Nitrogen* (gN/100g D.M.)		
	Cut 1	Cut 2	Cut 3
0	1.91	1.94	2.50
40	1.96	2.04	2.72
80	1.89	1.79	2.13
160	1.75	1.82	2.57
Standard error	± 0.11	± 0.12	± 0.16

Effect of S not significant at any cut.

(b) Dykegatehead

Added S (kgS/ha)	Nitrogen* (gN/100g D.M.)		
	Cut 1	Cut 2	Cut 3
0	1.48	1.99	2.45
40	1.52	2.06	2.45
80	1.59	2.08	2.48
160	1.89	2.12	2.44
Standard error	± 0.17	± 0.15	± 0.13

Effect of S not significant at any cut.

*mean of two replicate plots.

additions and no effect of added sulphur upon crude protein-N was consistent during the two growing seasons. Marked differences in crude protein-N content were seen between cuts at both sites and during both years with the levels increasing steadily throughout the season.

As no truly sulphur deficient herbage was grown it was unlikely that consistent relationships between sulphate and nitrate levels would emerge. Results (Tables 17 and 18) supported this and no evidence of nitrate accumulation in tissue low in sulphate was observed (correlation coefficients between nitrate and sulphate levels were only: $r = 0.10$ for 1977 data and $r = -0.14$ for 1978 data). Again differences between cuts were most evident with accumulation of nitrate during the latter part of the season. Therefore the lowest sulphur levels obtained in this work did not appear to limit the conversion of nitrate into crude protein-N.

4.2.1.3 Dry-matter production (Tables 19 and 20)

As would be expected from the values of total sulphur, which indicated sulphur sufficiency, no dry matter yield responses to added sulphur were obtained. The ryegrass crop was therefore, at present, able to obtain sufficient sulphur from soil and atmospheric sources. Yields varied between cuts; the first cut (the main silage cut) being the largest and subsequent cuts gradually decreasing. A shorter growing season in 1978 depressed yields and only three cuts could be made compared with the five made

TABLE 17. Nitrate-nitrogen content of herbage harvested during 1977.

(a) Blackaddermount

Added S (kgS/ha)	Nitrate-nitrogen* (µgN/g D.M.)				
	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5
0	150	525	970	2170	1120
5	460	610	1680	2580	1960
10	294	640	1330	3500	2780
20	266	690	1270	2780	2520
40	530	690	1050	3140	2840
80	150	480	1350	2060	720
160	76	330	820	2880	1280

Standard error of cut mean ± 175. Standard error of treatment mean ± 247. Effect of S significant ($P < 0.05$), effect of cut significant ($P < 0.001$)

(b) Dykegatehead

Added S (kgS/ha)	Nitrate-nitrogen* (µgN/g D.M.)			
	Cut 1	Cut 2	Cut 3	Cut 4
0	61	470	380	825
5	n.d.	970	310	1540
10	66	410	280	920
20	71	200	330	1180
40	110	110	360	500
80	28	440	150	480
160	190	1270	710	2480

Standard error of cut mean ± 119. Standard error of treatment mean ± 158. Effect of S significant ($P < 0.05$), effect of cut significant ($P < 0.001$)

*mean of duplicate determinations.

TABLE 18. Nitrate-nitrogen content of herbage harvested during 1978.

(a) Blackaddermount

Added S (kgS/ha)	Nitrate-nitrogen* (µgN/g D.M.)		
	Cut 1	Cut 2	Cut 3
0	370	645	2325
40	410	940	2785
80	465	550	1530
160	310	490	1765
Standard error	± 251	± 210	± 508

Effect of S not significant at any cut.

(b) Dykegatehead

Added S (kgS/ha)	Nitrate-nitrogen* (µgN/g D.M.)		
	Cut 1	Cut 2	Cut 3
0	225	895	1995
40	650	845	2340
80	140	1045	475
160	1355	955	1710
Standard error	± 307	± 353	± 651

Effect of S not significant at any cut.

*mean of two replicate plots.

TABLE 19. Dry-matter yields obtained from Dykegatehead during 1977.

Added S (kgS/ha)	Dry-matter yields (kg/ha)				
	Cut 1	Cut 2	Cut 3	Cut 4	Total
0	7886	4708	2713	1416	16723
5	7375	4500	2801	1599	16275
10	7431	4785	2650	1285	16151
20	7824	4277	2805	1419	16325
40	6915	4464	2585	1363	15327
80	7853	4083	2773	1474	16183
160	7480	4535	2693	1381	16089

Standard error of cut mean ± 85. Standard error of treatment mean ± 113. Effect of S not significant, effect of cut significant ($P < 0.001$)

TABLE 20. Dry-matter yields obtained during 1978.

(a) Blackaddermount

Added S (kgS/ha)	Dry-matter yield* (kg/ha)		
	Cut 1	Cut 2	Cut 3
0	5829	3983	3942
40	5935	3625	3626
80	6042	3725	3673
160	6042	4308	3587
Standard error	± 295	± 251	± 303

Effect of S not significant at any cut.

(b) Dykegatehead

Added S (kgS/ha)	Dry-matter yields* (kg/ha)		
	Cut 1	Cut 2	Cut 3
0	5775	4834	3176
40	6223	5134	2935
80	5611	5275	3463
160	5703	4850	2982
Standard error	± 337	± 324	± 290

Effect of S not significant at any cut.

*mean of two replicate plots.

during 1977. Whilst the yield data has not indicated a sulphur deficiency problem it is questionable whether or not the soil and atmosphere could continue to supply sulphur at rates needed for maximum yields in the future.

4.2.1.4 The sulphur budget

The amount of sulphur entering into the grassland situation from the atmosphere was measured and then subtracted from the amount of sulphur removed in the herbage at harvest. Estimation of the sulphur inputs and outputs enabled quantification of the sulphur contribution made by the soil organic sulphur pool. The concentration of sulphate in the rainfall at each site was determined monthly. Also daily rainfall measurements made at local weather stations at Tweedhill and Chirnside were recorded. These two pieces of information enabled calculation of the amount of sulphur contributed by the rainfall. Sulphur added by dry deposition and particulate deposition was determined as described above in section 3.2.1.3. The compound fertiliser used in 1977 was found to contain small quantities of sulphur and at the high fertiliser application rates used this contributed significant amounts of sulphur which were incorporated into the budget sheet (Table 21). All the atmospheric sulphur inputs were calculated for the period of the growing season rather than for the whole year. This is because sulphur deposited over the preceeding winter period would probably not be available to the grass plant in the spring. Evidence for this was provided by the soil

monitoring data (Figs. 2 and 3) which showed that calcium sulphate applied in the spring was completely removed from the topsoil before the following spring. This removal was probably due to leaching since it has been found that no significant incorporation of added sulphate occurred during winter (McLaren, 1976). The soil sulphate data also showed that levels of sulphate in the zero treated plots remained constant over the year. With this information and assuming that no sulphate leaching occurred during the growing season (unlikely since evapotranspiration rates invariably exceeded precipitation from April-October, in Berwickshire) one can be sure that the balance sheet is complete. For the larger additions of sulphur, some sulphate remained in the topsoil at the end of the growing season and therefore the sulphur surplus shown on the balance sheets was underestimated. Examination of the balance sheet is very interesting and shows that the soil contributed almost half of the sulphur taken up by the crop in 1977 and over half of that taken up in 1978 (Tables 22 and 23). Such a rate of sulphur mineralisation, representing 1.3% of the total soil sulphur during 1977, could also have simultaneously occurred where higher additions of sulphur had been made. However since a large excess to plant requirements was added, quantities of sulphur mineralised will be masked. One important omission from the budget sheet is the quantity of sulphur contained in the plant roots and stubble and hence the

Figure 2. Soil sulphate levels at Blackaddermount, 1977/78.

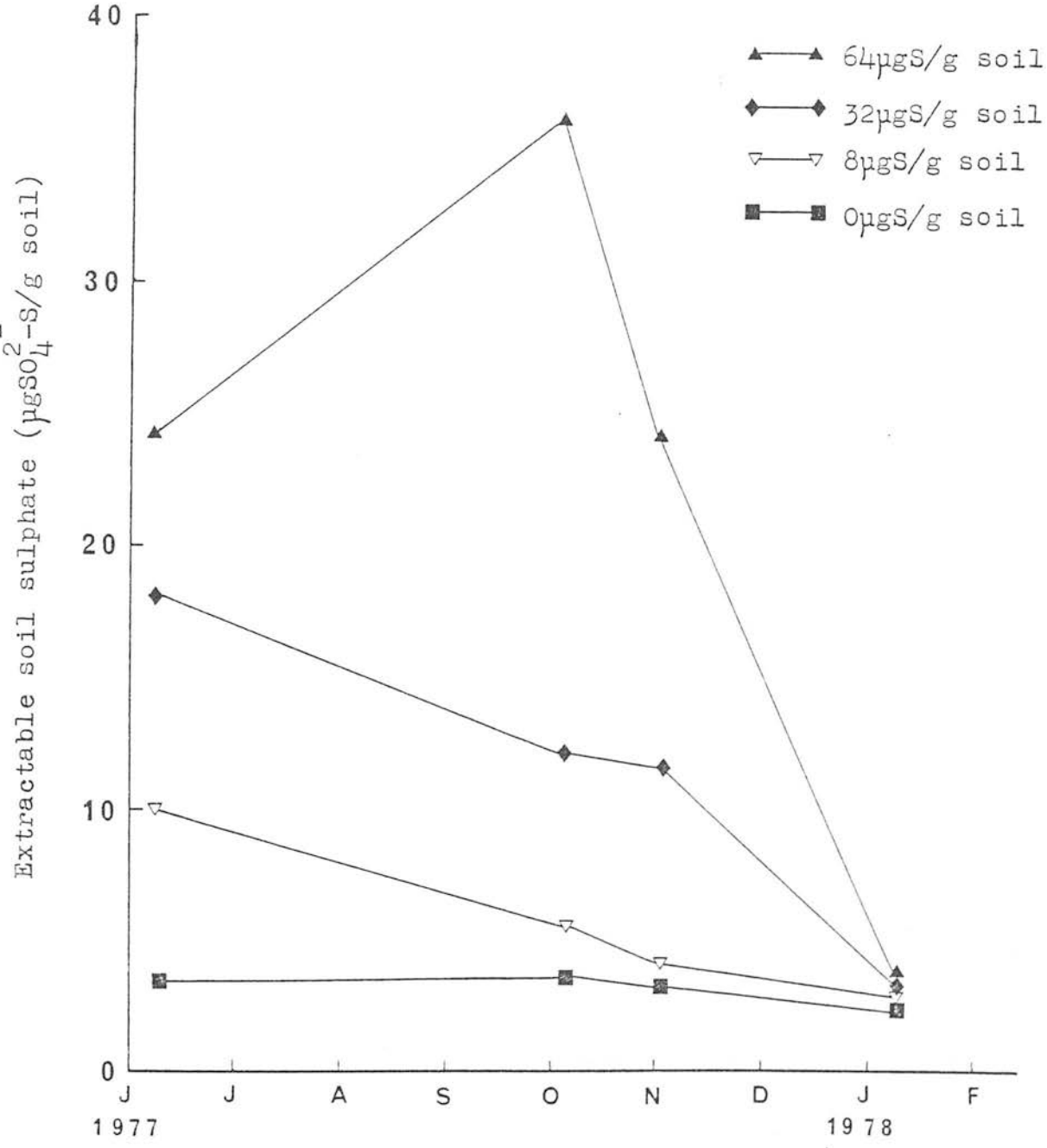


Figure 3. Soil sulphate levels at Dykegatehead 1977/78.

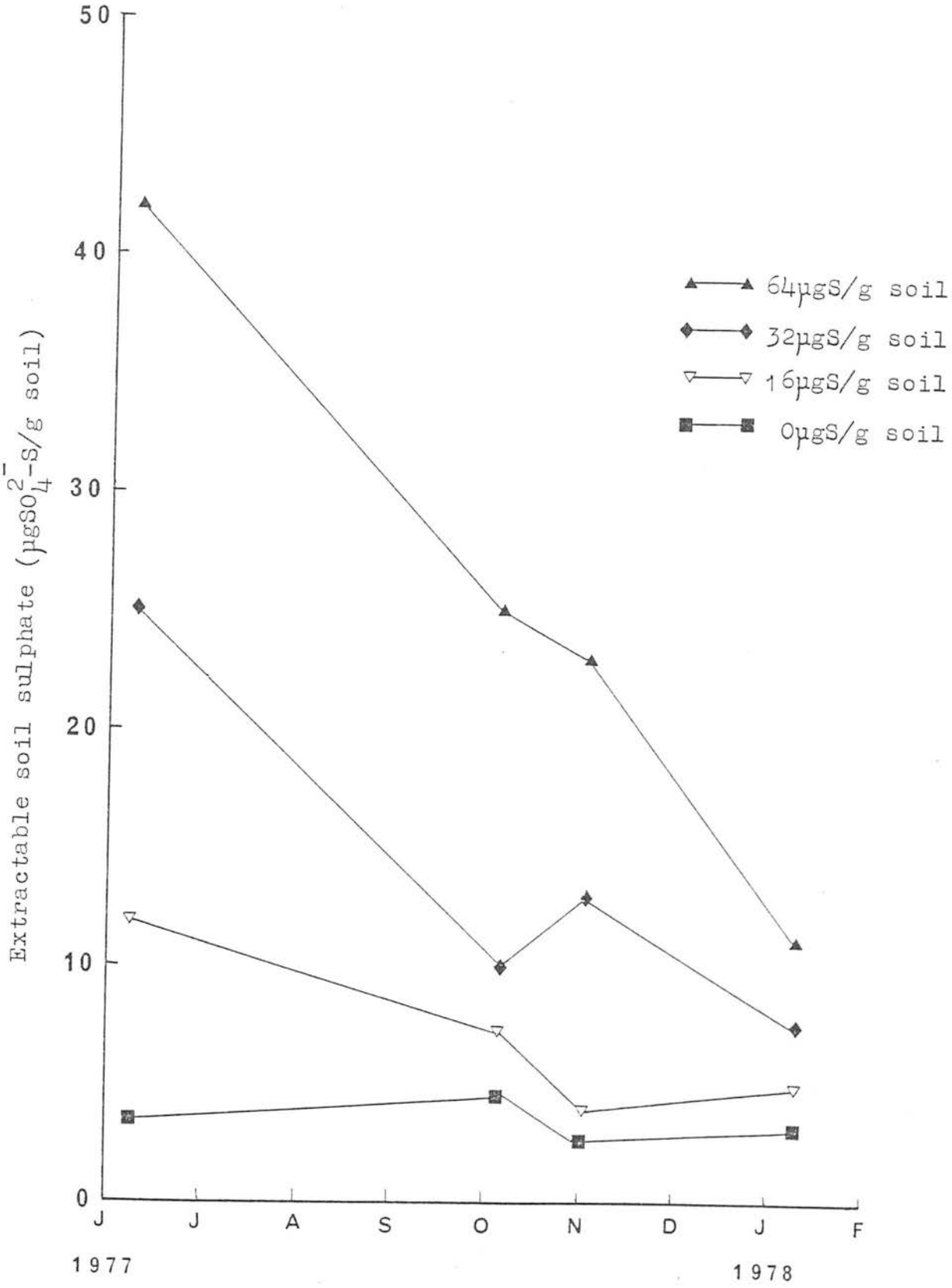


TABLE 21. The sulphur budget sheet for Dykegatehead from April → October 1977 (kgS/ha)

Sulphur Inputs						
Added Sulphur	Compound Fertiliser Sulphur	Dry Deposition	Particulate Deposition	Rainfall	Crop Removal	Balance
0	10	2.6	0.2	5.5	33.3	- 15.0
5	10	2.6	0.2	5.5	38.2	- 15.1
10	10	2.6	0.2	5.5	35.8	- 7.4
20	10	2.6	0.2	5.5	39.4	- 1.1
40	10	2.6	0.2	5.5	40.7	17.6
80	10	2.6	0.2	5.5	45.3	53.0
160	10	2.6	0.2	5.5	52.7	125.6

Values based on 1978 data.

TABLE 22. The sulphur budget sheet for Blackaddermount from April → October 1978 (kgS/ha)

	Sulphur Inputs				
Added Sulphur	Dry Deposition	Particulate Deposition	Rainfall	Crop Removal	Balance
0	2.6	0.2	6.8	28.0	- 18.4
40	2.6	0.2	6.8	33.8	15.8
80	2.6	0.2	6.8	39.5	50.1
160	2.6	0.2	6.8	38.2	131.4

TABLE 23. The Sulphur budget sheet for Dykegatehead from April → October 1978 (kgS/ha)

	Sulphur Inputs				
Added Sulphur	Dry Deposition	Particulate Deposition	Rainfall	Crop Removal	Balance
0	2.6	0.2	7.6	26.7	- 16.7
40	2.6	0.2	7.6	30.6	19.6
80	2.6	0.2	7.6	41.0	49.2
160	2.6	0.2	7.6	38.0	92.2

estimated amount of sulphur mineralised could be a slight under-estimate. During the 1978 season 1.5 percent of the total soil sulphur was again mineralised but some of this sulphur could have originated from sloughed off, dead root material from the previous season and not derived directly from the soil sulphur pool.

The budget sheet clearly shows the significance of sulphate derived from soil organic sulphur where atmospheric inputs alone were insufficient for maximum herbage production. This marginal situation necessitates a greater understanding of short term mineralisation/ immobilisation processes occurring in the soil. Also the more labile forms of organic sulphur, which are the precursors of available sulphate, need to be identified so that a measure of a soils potential to mineralise sulphur can be made. The need for a measure of the soils sulphur status, other than the widely used extractable sulphate, is clear when one considers that the extractable sulphate of the Whitsome soil was insufficient for the first cut of grass at both sites.

4.2.2 West of Scotland Field Trials

The field trials in the West of Scotland were set up after reports of low herbage sulphur contents in this area had been received (McPherson, 1978). The trials were designed to investigate plant yield response to added sulphur. Experimental details have been reported above (section 3.3.2).

4.2.2.1 Sulphur content of herbage

Additions of sulphur improved the sulphur content of herbage at both Woodhead and Crichton (Table 24). The treatment effect at Crichton was more pronounced, presumably because extractable soil sulphate levels were substantially lower than those at Woodhead. Herbage from plots which received no added sulphur had a sulphur content very close to values considered critical for optimum sulphur nutrition (0.15-0.16% S). Again herbage collected from Crichton showed the lower basal levels of sulphur because less extractable soil sulphate was present. As with the Berwickshire field trials, plant sulphate-S appeared to be a more sensitive indicator of plant sulphur status. Addition of sulphur caused a two and often three fold increase in herbage sulphate levels. Indeed much of the increase in total sulphur can be attributed to increased sulphate levels. Total sulphur values for the zero treatments indicated that a very marginal sufficiency existed at Crichton and yield responses to added sulphur might be expected. At Woodhead the herbage total sulphur contents were sufficient and no yield improvements brought about by sulphur additions would be expected.

4.2.2.2 Dry-matter production

The yield data (Table 24) exhibited no significant response to added sulphur. Hence the soil and atmosphere are presently supplying adequate sulphur for maximum yields in this part of Scotland. In addition

TABLE 24. Plant total-sulphur, plant sulphate-sulphur and dry matter production - West of Scotland field trials

(a) Crichton

Added S (kgS/ha)	CUT 1			CUT 2		
	D.M. Yield (kg/ha)	Total-S (gS/100g D.M.)	SO_4-S (µgS/g D.M.)	D.M. Yield (kg/ha)	Total S (gS/100g D.M.)	SO_4-S (µgS/g D.M.)
0	5058	0.18	413	3359	0.20	413
50	5061	0.29	1254	3500	0.23	712
Standard error	± 615	± 0.022	± 139	± 224	± 0.018	± 100
significance level*	N.S.	***	***	N.S.	*	**

*significance of difference between treatments:- N.S., not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

TABLE 24 (Contd.)

(b) Woodhead

Added S (kgS/ha)	CUT 1			CUT 2		
	D.M. Yield (kg/ha)	Total-S (gS/100g D.M.)	SO_4-S (μgS/g D.M.)	D.M. Yield (kg/ha)	Total S (gS/100g D.M.)	SO_4-S (μgS/g D.M.)
0	3898	0.23	649	3061	0.27	739
50	3528	0.27	1172	2928	0.31	1164
Standard error	± 466	± 0.023	± 138	± 219	± 0.015	± 109
significance level	N.S.	*	**	N.S.	**	**

The above data represents the mean of four replicate plots

widespread use of slurry in this area may preserve supplies of organic sulphur in the soil.

4.2.2.3 Extractable soil sulphate (Table 25)

The difference between the initial soil sulphate levels at the two sites has been mentioned earlier in context with the effects upon sulphur levels in the herbage of zero treated plots (section 4.2.2.1). Soil analysis at the second cut clearly showed depressed levels of sulphate at both sites. This implied that mineralisation rates did not match uptake rates. These findings contrasted with the Berwickshire data where Whitsome soil sulphate levels in the zero sulphur treatments did not vary significantly during the growing season. Unfortunately only two cuts could be made but a third cut could well have shown a yield response to added sulphur since a further depletion of the soil sulphur pool would have been made.

Soil sulphate levels for the sulphur treated plots can be seen to be perfectly adequate for future cuts of grass made in the same season.

No detailed sulphur budget was determined for this area but total plant sulphur and extractable soil sulphate data clearly indicated that again only marginal sulphur sufficiency existed (predominantly westerly winds in this rural area are likely to contribute below average amounts of atmospheric sulphur).

TABLE 25. Extractable soil sulphate levels - West of Scotland field trials

Added S (kgS/ha)	Crichton (μgSO_4^{2-}-S/g)		Woodhead (μgSO_4^{2-}-S/g)	
	Pre-treatment	Cut 2	Pre-treatment	Cut 2
0	4.1	2.2	7.2	2.9
50		12.9		15.4
Standard error		± 2.2		± 1.7
significance level		***		***

The above data represents the mean of four replicate plots.

4.2.3 Field Trials in the Edinburgh Area (Boghall Farm)

The trials in the Edinburgh area were laid down, as described above in section 3.3.3., to examine possible yield responses to added sulphur, investigate the sulphur status of the ryegrass crop, and to construct a sulphur budget sheet for the area. This would enable comparisons to be made between rural Berwickshire and the more industrialised Edinburgh area.

4.2.3.1 Sulphur levels in herbage

The total sulphur data (Table 27) indicates that a yield response to added sulphur would not be expected. This is because the control plots, during both years, yielded herbage containing between 0.19 and 0.27 percent sulphur. These levels of sulphur were adequate to ensure maximum yields. The addition of fertiliser sulphur significantly increased total plant sulphur levels for both growing seasons. An increase in plant sulphur levels during the 1979 growing season was observed (such an increase was also noted for the Berwickshire field trials) although during 1980 plant sulphur levels remained fairly constant. The plant sulphur levels recorded during 1980 were generally higher than those recorded during 1979. This was probably due to the residual value of the sulphur applied in spring, 1979 (see sub-soil sulphate levels in Table 30) and the larger atmospheric sulphur input during 1980 (see sulphur budget sheets for 1979 and 1980 - Tables 31 and 32).

TABLE 28. Extractable plant sulphate content of herbage harvested from Boghall*, 1979 (µgS/g D.M.)

Added sulphur (kgS/ha)	CUT 1	CUT 2
0	986	1046
10	1118	1152
20	1453	1219
40	1601	1707
60	1395	1735
80	1645	2193
100	1645	2163

*mean of three replicate plots.

Extractable plant sulphate levels were only determined during 1979 (Table 28). As above, the percentage of the plant sulphur occurring as sulphate increased as the fertiliser sulphur addition increased. The data for the control plots showed that over forty percent of the total sulphur occurred as sulphate indicating again that yield responses to added sulphur were unlikely.

4.2.3.2 Dry-matter production (Table 26)

As predicted by the plant sulphur levels discussed in the previous section no dry-matter yield responses to added sulphur were obtained. Therefore during 1979 and 1980 the herbage received sufficient sulphur from soil and atmospheric sources.

4.2.3.3 The sulphur budget

A sulphur budget for the grass crop grown at Boghall for two growing seasons was prepared (Tables 31 and 32) in the same manner as for the Berwickshire field experiments reported above (section 4.2.1.4). The errors and limitations of such a budget are fully discussed in section 4.2.1.4. Extractable soil sulphate levels showed that practically all the sulphur applied during the spring of 1979 was removed from the topsoil (0-20 cms) by March 1980 (as was found in Berwickshire during 1977 and 1978). However analysis of soil, sampled at depth, showed that where 100 kgS/ha had been added significant amounts of sulphur at 30-40 cms depth,

TABLE 26. Dry-matter yields obtained from Boghall, 1979 and 1980 (kg D.M./ha)

Added sulphur	1979		1980	
kgS/ha	CUT 1	CUT 2	CUT 1	CUT 2
0	6471	2871	5621	4056
10	7201	2884	5684	4337
20	6916	2919	5558	4007
40	7344	2531	5517	4197
60	7914	2842	6105	3979
80	7129	2862	5726	4309
100	8627	2943	6727	4748
Standard error	± 649	± 193	± 552	± 219

Effect of added sulphur was not significant at any cut.

*mean of three replicate plots.

TABLE 27. Total sulphur content of herbage harvested from Boghall*, 1979 and 1980 (gS/100g D.M.)

Added sulphur	1979		1980	
(kgS/ha)	CUT 1	CUT 2	CUT 1	CUT 2
0	0.186	0.265	0.226	0.265
10	0.199	0.284	0.270	0.277
20	0.241	0.285	0.253	0.287
40	0.243	0.340	0.276	0.273
60	0.231	0.329	0.290	0.305
80	0.259	0.382	0.282	0.286
100	0.255	0.365	0.345	0.290
Standard error	± 0.006	± 0.008	± 0.020	± 0.018

Effect of added sulphur was significant in 1979 for both cuts and for cut 1 in 1980 ($P < 0.01$) but not significant at cut 2 in 1980.

*mean of three replicate plots.

TABLE 29. Extractable soil sulphate levels at Boghall during 1979-1980 (μgSO_4^{2-}-S/g soil)

Added sulphur (kgS/ha)	Pre-fertilisation (5/4/79)	Cut 2[f] (5/10/79)	Pre-fertilisation[f] (14/3/80)
0	5.0[*]	2.7	6.0
10	5.0	3.8	5.9
20	5.0	4.5	6.6
40	5.0	8.5	7.4
60	5.0	11.7	7.8
80	5.0	13.7	8.8
100	5.0	16.1	7.6

[*]Analysis performed on a bulk sample collected from trial area.

[f]Mean of three replicate plots.

TABLE 30. Extractable sub-soil sulphate levels at Boghall during Spring 1980 (μgSO_4^{2-}S/g soil)[*].

Soil depth (cm.)	Added sulphur 0kgS/ha	100kgS/ha
0-10	7.5	8.9
10-20	6.3	7.0
20-30	4.2	7.5
30-40	4.6	12.8

[*]Mean of four replicate cores

was still present the following spring. Since this "residual"sulphate would be available to the grass crop the sulphur surplus shown on the 1980 budget sheet for the larger additions of sulphur was underestimated.

Examination of the budget sheets showed that even in an urban area of substantial atmospheric sulphur input the soil remained a net contributer of sulphur. In 1979 42 percent of the sulphur removed by the crop was derived directly from the soil whilst in 1980 34 percent was obtained from soil supplies. The application of 10kgS/ha appeared to almost balance sulphur input with crop removal and therefore in this case no depletion of the soil sulphur occurred. Where applications of sulphur greater than 10kgS/ha were made, large surpluses of sulphur were found indicating unnecessarily high rates of application. The balance sheets are very similar for the two growing seasons except that high summer rainfall during 1980 increased the amount of sulphur added in rainfall. Some variation in the dry deposition of sulphur between the two growing seasons was also apparent.

To make up the sulphur deficit of the control plots required mineralisation of 0.7 percent of the total soil sulphur during the 1979 growing season and 0.7 percent during the 1980 growing season. Laboratory incubation and sulphur-35 experiments described below (sections 4.4 and 4.5 respectively) indicate that such levels of sulphur mineralisation appear realistic.

TABLE 31. The sulphur budget sheet for Boghall from Apr. → Oct. 1979 (kgS/ha)

SULPHUR INPUTS					
Added Sulphur	Dry Deposition	Particulate Deposition	Rainfall	Crop Removal	Balance
0	4.68	0.13	6.6	19.64	- 8.23
10	4.68	0.13	6.6	22.52	- 1.11
20	4.68	0.13	6.6	24.99	6.42
40	4.68	0.13	6.6	26.46	24.95
60	4.68	0.13	6.6	27.63	43.78
80	4.68	0.13	6.6	29.39	62.02
100	4.68	0.13	6.6	32.74	78.67

TABLE 32. The sulphur budget sheet for Boghall from Apr. → Oct. 1980 (kgS/ha)

Added Sulphur	Dry Deposition	Particulate Deposition	Rainfall	Crop Removal	Balance
0	6.12	0.16	9.3	23.45	- 7.87
10	6.12	0.16	9.3	27.36	- 1.78
20	6.12	0.16	9.3	25.56	10.02
40	6.12	0.16	9.3	26.69	28.89
60	6.12	0.16	9.3	29.84	45.74
80	6.12	0.16	9.3	28.47	67.11
100	6.12	0.16	9.3	36.98	78.60

4.3 Pot Experiments

Although the field experiments provided valuable information on the sulphur budget, the time available permitted only two soil types to be thoroughly investigated. The pot experiments allowed a study of the sulphur status of several Scottish soil series. Also the more controlled conditions afforded by pot experimentation enabled an accurate investigation of the effects of added nitrogen and sulphur on the chemical composition of sulphur sufficient and sulphur deficient plants. The aims of the individual pot experiments are outlined below.

4.3.1 Experiment I

This experiment was set up using soils obtained from the two Berwickshire field sites. The aims were to investigate the differences in mineral composition between sulphur deficient and sulphur sufficient plant tissue. An attempt was also made to clarify why grass grown in the glasshouse showed yield responses to added sulphur (McLaren, 1975) whilst grass grown in the field did not.

4.3.1.1 Dry-matter production (Tables 33 and 34)

The first cut (52 days) showed no yield response to added sulphur but the effect of nitrogen additions could be clearly seen. Also little difference between soils was observed. The second cut, made after 87 days, showed a marked response to added sulphur where nitrogen had also been applied. The added sulphur increased dry matter yields by 25-36%. The control pots showed a stunted growth rate

TABLE 33. Pot Experiment I. Dry matter yields*

(g D.M./pot)

	Added Sulphur (mgmS/kg soil)	Blackaddermount Low N	Blackaddermount High N	Standard Error	Dykegatehead Low N	Dykegatehead High N	Standard Error
	0	4.9	6.3		4.2	6.7	
CUT 1	8	4.5	6.8	± 0.22	4.0	6.2	± 0.25
	40	4.6	6.8		3.9	7.1	
	0	-	3.9		-	4.6	
CUT 2	8	-	5.8	± 0.19	-	5.4	± 0.14
	40	-	6.1		-	6.1	
	0	4.6	3.2		4.2	3.7	
CUT 3	8	4.4	3.8	± 0.08	4.2	4.5	± 0.10
	40	4.4	4.4		4.2	4.5	

*mean of three replicate pots

The effect of added S not significant at cut 1 on either soil, significant on both soils at cut 2 and Blackaddermount at cut 3 ($P < 0.001$), and significant on Dykegatehead at cut 3 ($P < 0.01$)

The effect of added N significant on both soils at cut 1 and Blackaddermount at cut 3 ($P < 0.001$) and on Dykegatehead at cut 3 ($P < 0.001$).

TABLE 34. Pot Experiment I. Total dry matter yields for the growth period (g D.M./pot)

Added sulphur (mgS/kg soil)	Blackaddermount Low N	Blackaddermount High N	Dykegatehead Low N	Dykegatehead High N
0	9.3	13.4	8.4	15.0
8	8.9	16.4	8.2	16.1
40	9.0	17.3	8.1	17.5

and the grass was noticeably chlorotic. Consequently the control pots were not harvested at the second cut. At the third cut, made after 170 days, the yield response to added sulphur was less marked but still evident in the high nitrogen treatments. Apart from the overall response to added sulphur the pots receiving 100 kgS/ha yielded significantly more dry matter than those receiving 20kgS/ha. Thus the herbage grown in the pot, receiving 320kg/N, required more than 20kgS/ha in addition to the sulphate derived from the soil organic pool to ensure optimum yields. However the 1978 Berwickshire control plots, which received only 10kgS/ha (as rainfall and atmospheric sulphur inputs) in addition to soil sulphur did not give yield responses to added sulphur. Factors contributing to this disparity between field and pot experiments included higher plant population density in the pot and a small, shallower volume of soil which would become depleted of nutrients more quickly than in the field.

The yield data overall can be explained in terms of differential rates of sulphur supply versus rates of sulphur uptake. At the beginning of the experiment plants utilised sulphur derived from seed reserves and the readily available soil sulphate and therefore no yield responses would be expected at the first cut. However by the second cut, soil sulphate levels were depleted and where net sulphur mineralisation rates did not match plant requirement a response to added sulphur would be expected.

A less marked yield response to added sulphur might be expected at the third cut since the senescing grass required less sulphur.

4.3.1.2 Recognition of sulphur deficiency by plant analysis

This work utilised the herbage from the second cut as it contained examples of sulphur sufficiency and sulphur deficiency. Throughout this experiment plant total sulphur was determined by a chemical oxidation method which was shown to underestimate the amount of sulphur present (see Appendix II). Therefore total sulphur values can only be compared withinthe experiment and not with values obtained for the field trials. It was found that total sulphur values did not clearly relate to sulphur deficiency/sufficiency observations made at the various stages of growth. This was because total sulphur values for sulphur deficient and sulphur sufficient plants differed very little making assessment of the plants sulphur status difficult (Table 35).

Extractable plant sulphate levels exhibited an improved relationship with yield and are therefore probably more useful for the diagnosis of the sulphur status of herbage. From the data (Table 36 and Fig. 4) it can be seen that when levels of extractable plant sulphate fell below 600µgS/g a yield response to added sulphur could be expected. This compares with critical plant sulphate values of 100µgS/g reported by Freney et al., (1978) for wheat and 320µgS/g for ryegrass (Dijkshoorn et

TABLE 35. Pot Experiment I. Plant Total Sulphur*

(µgS/g D.M.)

	Added Sulphur (mgmS/kg soil)	Blackaddermount		Standard Error	Dykegatehead		Standard Error
		Low N	High N		Low N	High N	
	0	1693	667		2583	1493	
CUT 1	8	2793	1753	± 78	3197	2540	± 83
	40	3057	2887		3760	3230	
	0		960			967	
CUT 2	8		950	± 167		1163	± 144
	40		2187			2350	
	0	1880	800		2280	1000	
CUT 3	8	2300	1000	± 76	2800	1160	± 326
	40	3450	2440		4140	3750	

*duplicate determinations made on each of three replicate plots.

Effect of S significant at cut 1 on both soils and at cut 2 on Dykegatehead ($P < 0.001$), significant at cut 2 and cut 3 on Blackaddermount ($P < 0.01$), but not significant at cut 3 on Dykegatehead.

Effect of N significant at cut 1 on both soils ($P < 0.001$) and at cut 3 on Blackaddermount ($P < 0.01$), but not significant on Dykegatehead at cut 3.

TABLE 36. Pot Experiment I. Extractable plant sulphate* ($\mu g SO_4^{2-}$-S/g D.M.)

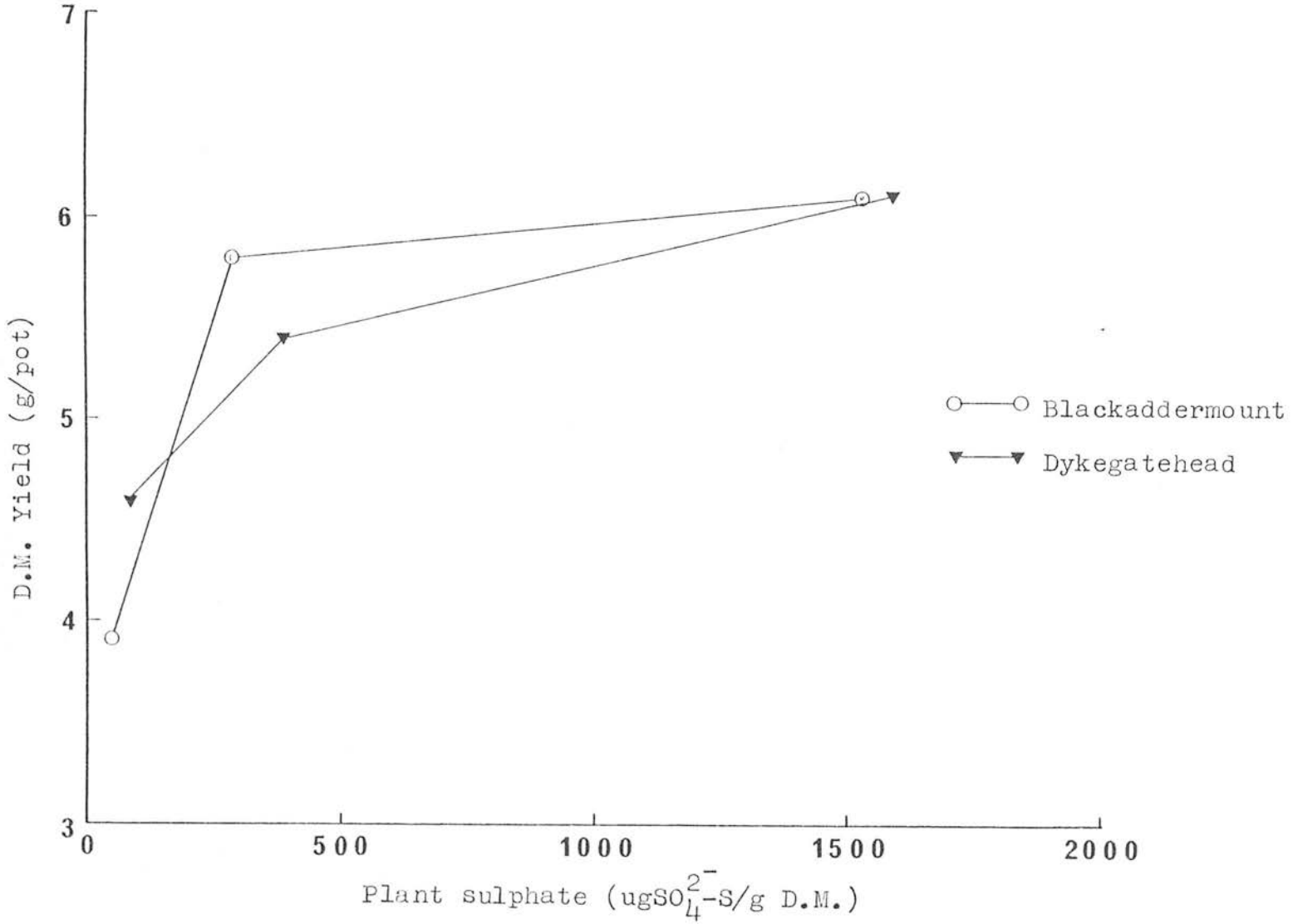
	Added Sulphur (mgmS/ kg soil)	Blackaddermount Low N	Blackaddermount High N	Standard Error	Dykegatehead Low N	Dykegatehead High N	Standard Error
CUT 1	0	950	320	± 59	1600	527	± 82
	8	1910	940		2033	1247	
	40	2227	1887		2520	1793	
CUT 2	0		50	± 88		87	± 135
	8		287			387	
	40		1593			1533	

*duplicate determinations on each of three replicate pots.

Effect of S significant at cut 1 and cut 2 on both soils ($P < 0.001$)

Effect of N significant at cut 1 on both soils ($P < 0.001$).

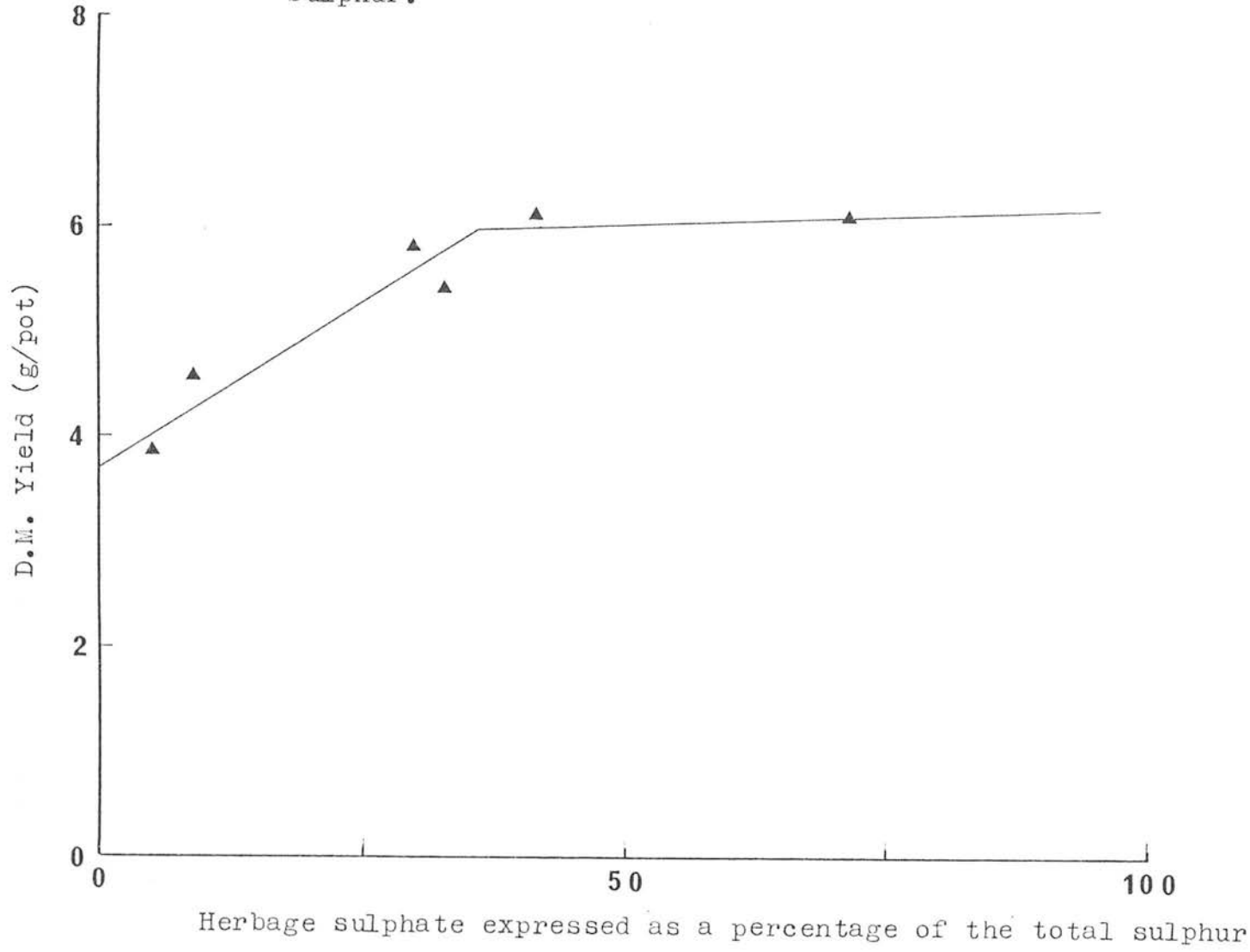
Figure 4. The relationship between plant sulphate levels and yield (cut 2, high N)



al., 1960.) The absence of a yield response to added sulphur at the Berwickshire field sites fits in with the above value since extractable sulphate levels in the herbage never fell below 600µgS/g. The fact that sulphate levels in herbage will represent a balance between uptake and utilisation (incorporation of sulphate into complex organic molecules) probably explains its usefulness in sulphur status diagnosis.

The best indication of herbage sulphur status was indicated by the sulphate content expressed as a percentage of the total sulphur. Other workers have reported similar findings (Freney et al., 1978). Herbage containing at least 30% of the total sulphur as sulphate was sulphur sufficient (Fig. 5) whereas herbage containing a lesser proportion of sulphate may show yield responses to added sulphur. This measurement of plant sulphur status is especially useful since it appears to be independent of plant age. This allows earlier recognition of sulphur deficiency in time for remedial action to be most effective. An investigation into the relationship between extractable plant sulphate and extractable plant nitrate in herbage was performed. It was thought that where sulphate contents were low, a limitation on protein manufacture within the plant might be imposed, which would be indicated by a build up of nitrate in the plant tissue. Results showed (Table 37) that any appreciable accumulation of nitrate only occurred when the extractable sulphate levels fell to

Figure 5. The use of herbage sulphur content expressed as a percentage of the total sulphur (cut 2, high N) for predicting D.M. yield responses to added sulphur.



around 50µgS/g. Since extractable plant sulphate levels are unlikely to fall to such low levels in the field, where atmosphere and rainfall additions occur, the accumulation of nitrate would appear to have little practical agricultural significance in the U.K. No overall correlation could be seen between the extractable sulphate and nitrate levels when sulphate levels exceed 50µgS/g ($r = -0.50$).

4.3.1.3 The nutritional quality of sulphur deficient/ sufficient herbage.

The level of total sulphur needed for optimum sulphur nutrition by livestock in between 0.18 and 0.25% S (Tisdale, 1977). However the plant total sulphur values reported here are underestimated and cannot be compared with other work which employed other methods of total sulphur determination.

The crude protein data (Table 38) provides no evidence for supposing that sulphur additions improved crude protein levels in herbage. No significant increases in crude protein were observed for either soil or either level of nitrogen addition at any cut. The data supported observations made above (section 4.2.1.2) about nitrate levels in herbage. Since sulphate levels were sufficient to prevent nitrate accumulation (except for S = 0µg/g soil and high N in CUT 2) then presumably they were also sufficient for optimum crude protein production. Although sulphur additions increased overall crude protein production, by increasing dry matter yields, no

TABLE 37. Pot Experiment I. Extractable plant nitrate*

(µgN/g D.M.)

	Added Sulphur (mgmS/kg soil)	Blackaddermount Low N	Blackaddermount High N	Standard Error	Dykegatehead Low N	Dykegatehead High N	Standard Error
	0	32	39		22	29	
CUT 1	8	32	35	± 3.8	17	36	± 5.1
	40	28	27		23	36	
	0		96			41	
CUT 2	8		62	± 7.7		39	± 4.8
	40		47			33	

*mean of duplicate determinations on each of three replicate pots.

Effect of S not significant on either soil at cut 1 or on Dykegatehead at cut 2, but significant at cut 2 on Blackaddermount ($P < 0.01$).

Effect of N not significant on Blackaddermount at cut 1 or Dykegatehead at cut 2, but significant at cut 1 on Dykegatehead at cut 2 on Blackaddermount ($P < 0.01$).

TABLE 38. Pot Experiment I. Plant Kjeldahl-nitrogen*

(gN/100g D.M.)

	Added Sulphur (mgmS/kg soil)	Blackaddermount Low N	Blackaddermount High N	Standard Error	Dykegatehead Low N	Dykegatehead High N	Standard Error
	0	1.39	1.60		1.29	1.37	
CUT 1	8	1.40	1.52	± 0.02	1.28	1.42	± 0.05
	40	1.37	1.59		1.43	1.37	
	0		1.83			1.40	
CUT 2	8		1.20			1.18	
	40		1.12			1.14	
	0	1.34	1.96		1.34	1.62	
CUT 3	8	1.30	1.59	± 0.14	1.34	1.40	± 0.05
	40	1.36	1.39		1.26	1.40	

*mean of duplicate determinations on each of three replicate pots.

Effect of added S not significant at any cut.

Effect of added N only significant at cut 1 on Blackaddermount ($P < 0.05$).

evidence for increased herbage crude-protein concentrations was obtained, which contrasted with previous work (McLaren, et al., 1978).

It was interesting to note that added nitrogen greatly affected both the levels of total sulphur and extractable sulphate in the plant tissue and the total amount of sulphur removed by the three harvests (Table 39(a)). Where no sulphur had been applied the high addition of nitrogen while increasing dry matter yields, reduced the amount of sulphur taken up over the growth period. When 20kgS/ha was applied similar amounts of sulphur were removed by the herbage at both high and low nitrogen levels.

However when 100 kgS/ha was applied greater amounts of sulphur were removed by the herbage when the high nitrogen addition was also made. These findings indicated that the plant did not discriminate between the nitrate and sulphate anions and therefore plant uptake varies in proportion to the relative abundance of the two nutrients. However when 100kgS/ha and the high nitrogen treatments are applied a positive interaction occurs and more sulphur is taken up than in the S=100kg/ha and low nitrogen treatment. McLaren (1976) similarly observed that herbage sulphur concentrations were reduced by nitrogen applications.

The realisation that nitrogen additions could decrease sulphur levels in herbage may have agricultural significance and also explains why other workers (Cowling

and Jones, 1970) have only observed yield responses to added sulphur in the presence of added nitrogen.

Although both soils contained similar amounts of extractable sulphate and total sulphur, the herbage from Dykegatehead consistently showed higher levels of total sulphur which is reflected in the values for uptake over the growing period. This observation cannot readily be explained since both soils displayed very similar chemical and physical properties. Such a difference between the soils was not seen in the field trial work.

4.3.1.4 The sulphur budget for the pot experiment

Since the pots will receive insignificant amounts of atmospheric sulphur (as sulphur dioxide and particulate sulphur) a rough budget sheet can be drawn up for the pot experiment (Table 39). Also the pots received no rainfall sulphur and therefore the sole remaining sources of sulphur were the soil and fertiliser sulphur. Extractable soil sulphate levels for the 0 and 12mgS/pot (20kgS/ha) treatments, determined at the third cut, had fallen to the original pre-fertilisation levels. This observation inferred that the sulphur required to balance removal at harvest originated from the organic soil sulphur pool. Soil sulphate values for the highest sulphur addition were very variable between replicates and therefore values for S = 60mg /pot (S = 100kg/ha) treatments in Table 39 do not take into account sulphate remaining in the soil.

TABLE 39. Pot Experiment I. The Sulphur Budget.

(a) Sulphur removed in herbage (mg S/pot)

Applied Sulphur (mg S/pot)	Blackaddermount		Dykegatehead	
	Low N	High N	Low N	High N
0	16.57	10.51	28.86	11.68
12	22.69	21.23	24.55	27.25
60	29.18	41.88	32.05	53.49

(b) Sulphur balance sheet (mgmS/pot) - indicating change in soil sulphur status after harvesting of herbage.

Applied Sulphur (mg S/pot)	Blackaddermount		Dykegatehead	
	Low N	High N	Low N	High N
0	- 16.57	- 10.51	- 28.86	- 11.68
12	- 10.69	- 9.23	- 12.55	- 15.25
60	30.82	18.12	27.95	6.51

(c) The percentage of the total soil sulphur mineralised to balance sulphur removed in the herbage.

Applied Sulphur (mg S/pot)	Blackaddermount		Dykegatehead	
	Low N	High N	Low N	High N
0	2.7	1.7	3.8	1.5
12	1.7	1.5	1.6	1.9
60				

Table 39(b) and (c) shows amounts of sulphur mineralised over the growing season. These amounts cannot be compared with amounts determined in the field because a chemical oxidation method was used which underestimated plant total sulphur. Between 7 and 11mg S/pot of sulphur has been mineralised where the two lower additions of sulphur have been made. Such a rate of net mineralisation could also have occurred in the highest sulphur treatments but no extractable soil sulphate data was available to substantiate this supposition.

The percentage of the total soil sulphur mineralised over the growing period (Table 39(c)) was markedly greater for the zero sulphur treatments receiving low nitrogen additions. All the other values fell within a narrow range with a mean value of 1.65%.

The much larger amounts of sulphate, made available by net mineralisation, in the S=0, Low N treatments could be a reflection of the greater amounts of sulphur removed by this treatment combination at harvest. Another explanation is that mineralisation rates are fairly constant for all treatments but where plant demand is high the sulphate is taken up rather than immobilised back into organic sulphur (work with S-35, reported in sections 4.5.1 and 4.5.2. demonstrates that net mineralisation vastly underestimated gross mineralisation).

4.3.2 Experiment II

This experiment was set up to assess and compare the sulphur status of nine agricultural soils from the East of

Scotland (for description see section 3.1.2). The sulphur status of each soil was investigated by:-

(i) examining dry-matter yield response to added sulphur.

(ii) calculating the amount of sulphur mineralised over the growing period.

(iii) determining the chemical properties of the soil (viz. organic matter, HI-reducible sulphur, readily mineralisable sulphur, total sulphur, extractable sulphate and pH).

4.3.2.1 Dry-matter production (Table 40)

A pattern of yield response to added sulphur, similar to that found in the previous pot experiment, also emerged in this experiment. However because the treatments were only replicated twice very few of the yield responses were statistically significant (Macmerry at cuts 1 and 2). Again the largest response to added sulphur occurred at the second cut. However some soils gave yield responses at the first cut whilst the third cut showed only small differences between the zero and 40 µgS/g soil (100kgS/ha) sulphur treatments. All the soils except Boyndie and Biel gave some yield response to added sulphur at cut 2, the most marked being Macmerry (29% yield increase), Eckford (20%), Hobkirk (17%), Darvel (14%) and Humbie (13%). The percentage yield increase at the first cut is significantly correlated with the original extractable soil sulphate levels ($r = -0.55^{*}$). This shows that extractable

TABLE 40. Pot Experiment II. Dry-matter yields* (g/pot)

Soil series	Added sulphur (mg S/kg soil)	CUT 1	CUT 2	CUT 3	TOTAL
Eckford	0	3.05	2.85	2.10	8.00
Eckford	40	3.30	3.50	2.50	9.00
Hobkirk	0	3.95	3.45	2.30	9.70
Hobkirk	40	4.30	4.15	2.45	10.90
Biel	0	5.60	5.40	3.25	14.25
Biel	40	5.76	5.20	3.25	14.20
Pow	0	5.10	4.65	3.55	13.30
Pow	40	5.70	5.10	3.35	14.15
Macmerry	0	3.55	2.90	2.45	8.90
Macmerry	40	4.45	4.10	2.75	11.30
Darvel	0	5.10	4.10	2.70	11.90
Darvel	40	5.30	4.75	3.10	13.15
Sourhope	0	2.65	3.85	2.30	8.80
Sourhope	40	2.50	4.30	2.50	9.30
Boyndie	0	5.75	4.85	3.25	13.85
Boyndie	40	5.60	5.20	3.50	14.30
Humbie	0	4.30	3.00	2.25	9.55
Humbie	40	4.20	3.90	2.55	10.65
Standard error		± 0.28	± 0.40	± 0.15	

Effect of S significant on Macmerry at both cut 1 and cut 2 ($P < 0.05$)

*mean of two replicate pots

soil sulphate is a good indicator of the sulphur, immediately available to plants. However the soil sulphate values also correlate with percentage yield increases at cut 2 ($r = -0.73^{**}$) even though all the original soil sulphate would be removed by the herbage harvested at the first cut (for the responsive soils only). This suggested that extractable soil sulphate levels could also be used to predict amounts of labile sulphur present in the soil. It was not surprising to find that the Boyndie and Biel soils showed no yield response to added sulphur as they contained 25.3 and 17.2 μgS/g soil of extractable soil sulphate respectively. The difference in dry matter production between soils will be due to a combination of factors such as pH, nutrient status and soil physical conditions.

4.3.2.2 Chemical composition of the herbage

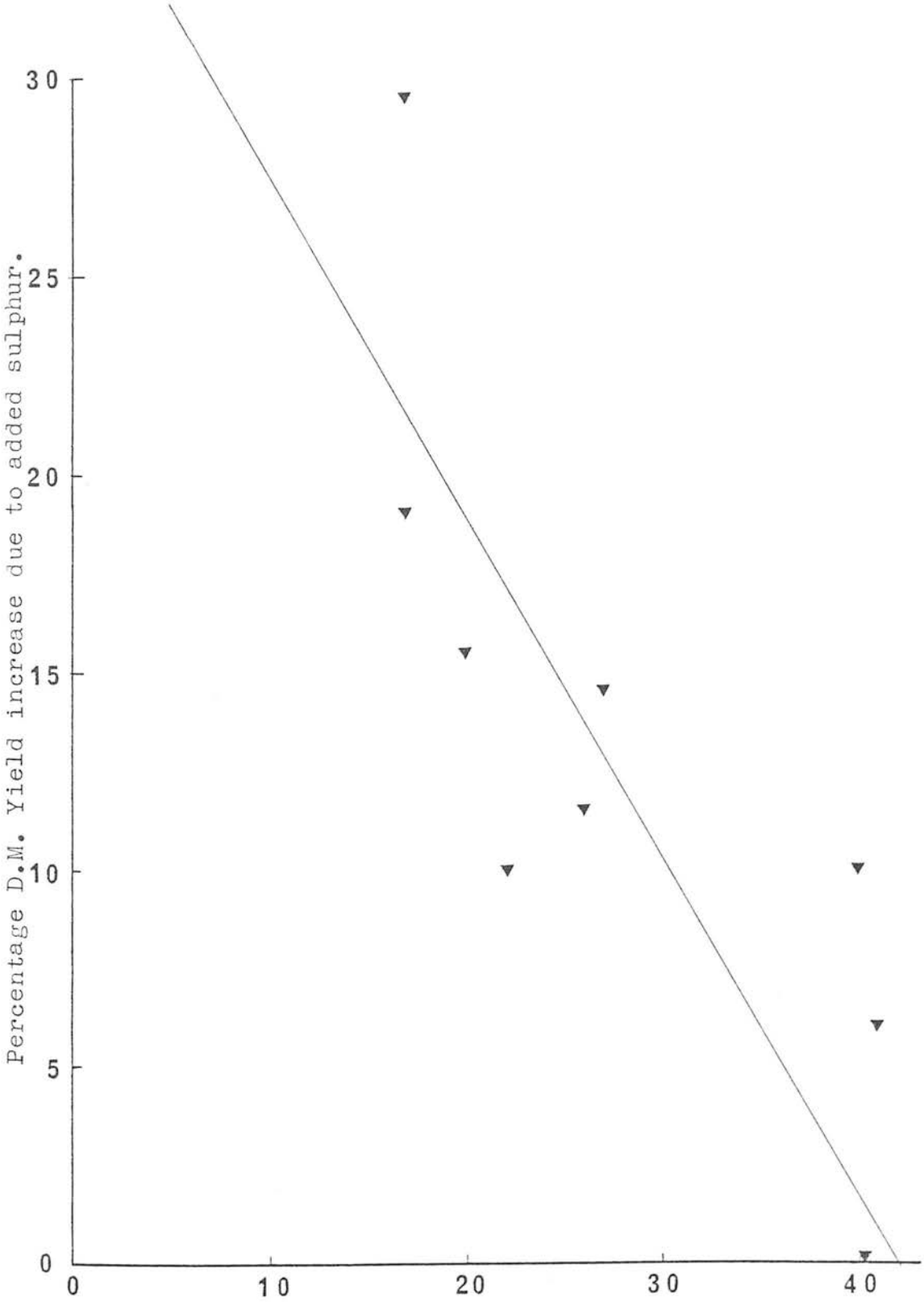
The total sulphur levels of the herbage in this experiment are unusually high (Table 41). Values are much higher than those found in the field. Indeed the total sulphur contents of the herbage, especially at the first two cuts, would suggest that no yield response to added sulphur was likely. However the previous pot experiment showed that total sulphur values were not an accurate predictor of sulphur sufficiency/deficiency. Again total S values did correlate with yield responses in this experiment but no basal value of total sulphur seems universally able to predict sulphur deficiency. Again the extractable plant sulphate proved the most

sensitive measure of a plants sulphur status - levels of 600-700 µgS/g herbage responded to added sulphur. The extractable plant sulphate expressed as a percentage of the total sulphur was another parameter which proved useful for assessing the sulphur status of the plant (Table 43). As with the field trials and previous pot experiment a yield response to added sulphur could be expected when the percentage of the total sulphate occurring as sulphate fell to below 25-30 percent. Again this value accurately indicated response independently of plant age. The addition of 60mg S/pot (equivalent to 100kgS/ha) increased the percentage of the total plant sulphur occurring as sulphate to between 43 and 64 percent for the first cut and maintained these levels for the remaining cuts (except for the Sourhope soil). Comparison of the percentage yield increases with the percentage of the total plant sulphur occurring as sulphate (Fig. 6) clearly demonstrated the usefulness of this parameter. For example the data predicted yield responses for the Eckford, Hobkirk and Macmerry soils at the first two cuts but not at cut three. It also suggested that yield responses at any cut were unlikely with the Boyndie and Biel soils. All these predictions were borne out by the yield data.

4.3.2.3 Sulphur mineralisation over the growing period (Table 44).

The amount of sulphur derived from the organic soil sulphur pool during the growth period was estimated, as

Figure 6. The prediction of yield response to added sulphur using plant extractable sulphate expressed as a percentage of the total plant sulphur (Pot Experiment II, cut 2).



previously, by measuring soil sulphate levels before and after growth, taking into account the sulphur treatments and measuring the amounts of sulphur removed in the herbage. Again small atmospheric sulphur inputs have been omitted as were the amounts of sulphur locked up in root tissue.

The data, as presented in Table 44, did not give estimates of gross sulphur mineralisation but quantities of sulphate derived from the soil organic pool (that is, net mineralisation). Net mineralisation occurred in all soils where no sulphur additions (21.7 — 39.0 mgS/pot) had been made and in all soils where sulphur had been added (4.6 — 14.4 mg S/pot) except for the Eckford and Hobkirk soils. The addition of 60 mg S/pot (100kgS/ha) greatly reduced the net mineralisation of sulphur. This large addition of sulphur ensured sufficiency for both plants and the micro-organisms responsible for immobilisation. However when soil sulphate levels were low, as in the case of the control pots; there was more competition between the plant roots and the soils microflora for the available sulphur. This competition had the effect of decreasing sulphate immobilisation and therefore also increased values of net mineralisation (where no sulphur was applied). It is unlikely that this reduction in mineralised sulphur is due to the sulphur addition directly affecting the soil micro-organisms responsible for sulphur mineralisation (incubation studies in sections 4.4.4 and 4.4.6 show that added sulphate does not affect net mineralisation of sulphur).

TABLE 41. Pot Experiment II. Total plant sulphur[/]

(gS/100g D.M.)

Soil series	Added sulphur (mg S/kg soil)	CUT 1	CUT 2	CUT 3
Eckford	0	0.381	0.465	0.199
Eckford	40	0.693***	0.709**	0.384***
Hobkirk	0	0.324	0.406	0.210
Hobkirk	40	0.406*	0.585*	0.309*
Biel	0	0.399	0.529	0.312
Biel	40	0.421	0.743**	0.405*
Pow	0	0.396	0.426	0.228
Pow	40	0.424	0.840***	0.542***
Macmerry	0	0.307*	0.426	0.225
Macmerry	40	0.387	0.658**	0.382**
Darvel	0	0.394	0.477	0.296
Darvel	40	0.616***	0.706**	0.393***
Sourhope	0	0.394	0.603	0.243
Sourhope	40	0.526***	0.660	0.274
Boyndie	0	0.476	0.560	0.234
Boyndie	40	0.511	0.698	0.462***
Humbie	0	0.353	0.452	0.247
Humbie	40	0.530***	0.828***	0.352*
Standard error		± 0.023	± 0.052	± 0.030

Significance levels:- * = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

[/] mean of two replicate pots.

TABLE 42. Pot Experiment II. Extractable plant sulphate[/]

(gS/100g D.M.)

Soil series	Added sulphur (mg S/kg soil)	CUT 1	CUT 2	CUT 3
Eckford	0	0.059	0.075	0.064
Eckford	40	0.363***	0.394***	0.252***
Hobkirk	0	0.070	0.073	0.082
Hobkirk	40	0.187***	0.278***	0.182**
Biel	0	0.156	0.213	0.160
Biel	40	0.185	0.491***	0.291***
Pow	0	0.150	0.167	0.113
Pow	40	0.188	0.525***	0.391***
Macmerry	0	0.048	0.070	0.080
Macmerry	40	0.165***	0.335***	0.236***
Darvel	0	0.155	0.172	0.152
Darvel	40	0.392***	0.405***	0.289***
Sourhope	0	0.160	0.131	0.028
Sourhope	40	0.259***	0.208	0.072
Boyndie	0	0.211	0.225	0.101
Boyndie	40	0.276*	0.276	0.312***
Humbie	0	0.088	0.116	0.086
Humbie	40	0.248***	0.494***	0.259***
Standard error		± 0.017	± 0.033	± 0.021

Significance levels * = P 0.05

** = P 0.01

*** = P 0.001

[/] mean of two replicate pots.

TABLE 43. Pot Experiment II. Extractable plant sulphate, expressed as the percentage of the total sulphur

Soil series	Added sulphur (mg S/kg soil)	CUT 1	CUT 2	CUT 3
Eckford	0	15	16	32
Eckford	40	53	56	66
Hobkirk	0	22	18	38
Hobkirk	40	46	48	59
Biel	0	39	40	52
Biel	40	44	66	72
Pow	0	38	40	49
Pow	40	45	63	72
Macmerry	0	16	17	36
Macmerry	40	43	51	62
Darvel	0	40	27	51
Darvel	40	64	57	75
Sourhope	0	42	22	11
Sourhope	40	49	32	26
Boyndie	0	44	41	43
Boyndie	40	54	60	68
Humbie	0	25	26	35
Humbie	40	47	60	75

In all cases soil sulphate levels decreased during the growing season and net mineralisation of sulphur was insufficient to maintain the original sulphate levels. This decrease was expected since net mineralisation provided insufficient sulphur for maximum plant yields in most of the soils. The Biel, Boyndie and Sourhope soils mineralised most sulphur in the control pots (although differences between the soil series were not marked) and these three soils exhibited the least yield response to added sulphur. In the case of added sulphur, again the amounts of sulphur mineralised differed only slightly between soil series except that the Eckford and Hobkirk soils both showed no net mineralisation. This was possibly because these two soils possessed wider C:S ratios than the other soil series (Table 45).

4.3.2.4 The relationship between sulphur mineralisation and some chemical properties of the soils

The amount of sulphur mineralised in each soil was expressed both as mg S/pot and as a percentage of the total soil sulphur. These values are shown together with several soil chemical parameters in Table 45. The percentages of the total soil sulphur mineralised are greater in this experiment than the field trials. However more sulphur was removed by the herbage during this experiment. Calculation of correlation coefficients showed that tentative relationships can be made, but using a small sample size of nine soils, one data point can greatly influence the value of the coefficient.

TABLE 44. Pot Experiment II. Changes in soil sulphate levels, plant uptake of sulphur and the sulphur derived from the soil organic pool (mg S/pot)

Soil series and sulphur addition*	Original soil sulphate	Final soil sulphate	Original minus final soil sulphate	Plant uptake of sulphur	Sulphur derived from soil organic pool/
Eckford	8.3	1.8	6.5	29.1	22.6
Eckford + S	68.3	9.0	59.3	56.1	- 3.2
Hobkirk	9.6	2.9	6.7	31.6	24.9
Hobkirk + S	69.6	19.7	49.9	49.6	- 0.3
Biel	25.8	3.8	22.0	61.0	39.0
Biel + S	85.8	18.6	67.2	73.6	6.4
Pow	29.4	2.4	27.0	48.7	21.7
Pow + S	89.4	12.1	77.3	84.9	7.6
Macmerry	9.9	3.4	6.5	28.8	22.3
Macmerry + S	69.9	19.8	50.1	54.7	4.6
Darvel	15.9	2.9	13.0	39.3	26.3
Darvel + S	75.9	11.8	64.1	78.5	14.4
Sourhope	32.1	28.1	4.0	38.3	34.3
Sourhope + S	92.1	54.1	38.0	48.8	10.8
Boyndie	38.0	2.6	35.4	62.0	26.6
Boyndie + S	98.0	24.1	73.9	81.1	7.2
Humbie	12.6	3.0	9.6	34.3	24.7
Humbie + S	72.6	15.6	57.0	63.8	6.8

* + S = 60 mgmS/pot

/ a negative value indicates no net mineralisation of sulphur

TABLE 45. Pot Experiment II. A comparison between the amount of sulphur mineralised and some soil chemical properties

Soil series	% of Total S mineralised in zero S treatment	% of Total S mineralised in + S treatment	Mineralised S from incubation* (µgS/g soil)	HI-reducible sulphur (µgS/g soil)	Total S µgS/g soil	Organic Matter (%)	Extr. SO_4^{2-}S (µgS/g)	C:S ratio	pH
Eckford	3.37	0	8.2 (1.84)	166	445	10.27	5.5	133	5.5
Hobkirk	4.15	0	7.0 (1.75)	125	400	6.84	6.4	98	5.8
Biel	5.76	0.95	7.8 (1.73)	183	450	4.92	17.2	63	7.4
Pow	4.54	1.25	6.8 (1.68)	160	405	3.39	19.6	48	7.1
Macmerry	2.78	0.56	7.8 (1.46)	157	535	5.36	6.6	58	5.9
Darvel	5.79	3.15	5.2 (1.72)	99	303	3.58	10.6	68	5.1
Sourhope	4.08	1.31	8.1 (1.45)	234	560	6.65	21.4	68	4.2
Boyndie	7.58	2.03	5.8 (2.49)	99	233	3.11	25.3	77	5.4
Humbie	4.49	1.22	8.3 (2.27)	122	365	3.83	8.9	60	7.3

*Net mineralised sulphate from 14 days incubation at 30°C - figures in parenthesis indicate the percentage of the total soil sulphur mineralised.

The percentage of the total soil sulphur mineralised by the control pots correlated with the percentage of the total soil sulphur mineralised by the pots receiving 60mg S/pot (100 kgS/ha) ($r = 0.667^{*}$). This indicated that although the added sulphur indirectly decreased net mineralisation of sulphur the relative capacity of each soil to mineralise sulphur remained unaffected. It was thought likely that a measure of the sulphur mineralised over the growing period would correlate with a value of net mineralisation obtained by an incubation method. However no correlation was obtained and the short term incubation technique might not provide a good estimate of the soil sulphur made available to plants over a season. However the amount of sulphate produced by net mineralisation during the incubation correlated with HI-reducible sulphur ($r = 0.67^{*}$) and total sulphur (0.743^{*}). The only other significant correlation was found between HI-reducible sulphur and total sulphur. This excellent correlation between total soil sulphur and mineralised sulphate is evident in work reported in section 4.4.7.

4.4 Laboratory Incubation Experiments

The previous sections (field trials and pot experiments) clearly showed that in three separate areas of Scotland soil sulphur contributions were essential to ensure sulphur sufficiency in grass, (where no additional sulphur was applied). In Scottish soils nearly all the sulphur occurs as unavailable organic compounds which require microbial mineralisation to convert the sulphur into plant available

forms. Therefore the rate of sulphur mineralisation determines whether or not grass crops remain sulphur sufficient in this area. Consequently sulphur mineralisation and the factors which affect it forms an important research subject. This section, using incubation techniques, seeks to evaluate the effect of time, glucose-carbon, temperature, sulphate and nitrate on sulphur mineralisation and attempts to correlate simple soil chemical parameters with sulphur mineralisation . The incubation approach does not improve our understanding of the reactions or compounds involved in the mineralisation of sulphur and overlooks the balance and complexity of mineralisation/immobilisation processes. However the technique is well suited for examining the effect of various factors by measuring changes in extractable sulphate - the key to crop sulphur sufficiency.

All incubation experiments were set up as described in section 3.5. Extractable soil sulphate was measured before and after a period of incubation and therefore results are expressed as net mineralised sulphate ($\mu g SO_4^{2-}$-S/g soil), except where indicated.

4.4.1 The effect of time on net sulphur mineralisation

The Linhope series soil and a calcareous pelosol were incubated at 30°C for the following lengths of time; 0, 1, 2, 3, 7, 14, 28, 42, 56 and 84 days. Figures 7 and 8 show net mineralised sulphate plotted against time, Both soils exhibited a very high initial rate of net

Figure 7. The rate of net sulphur mineralisation in Linhope series soil.

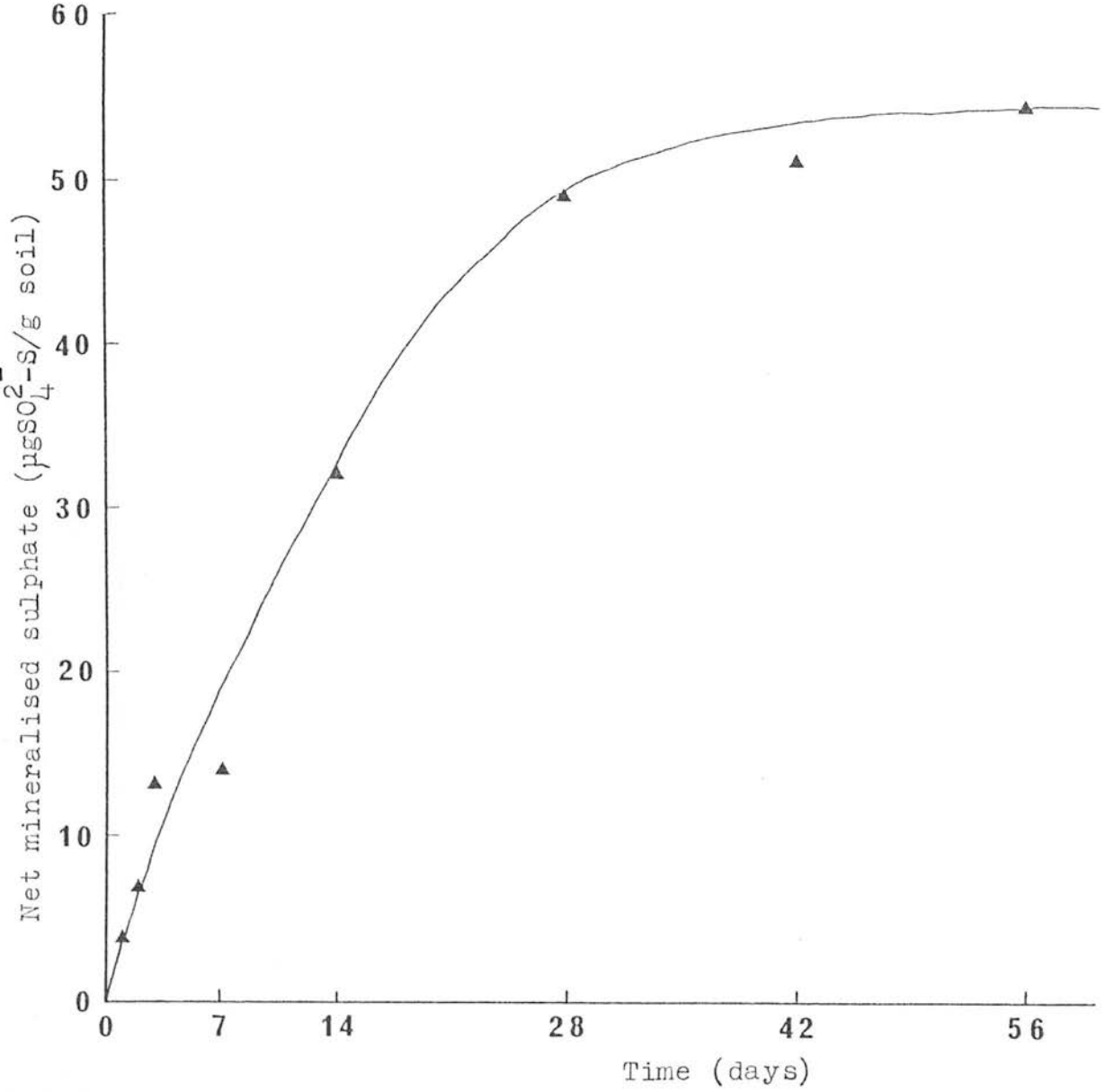
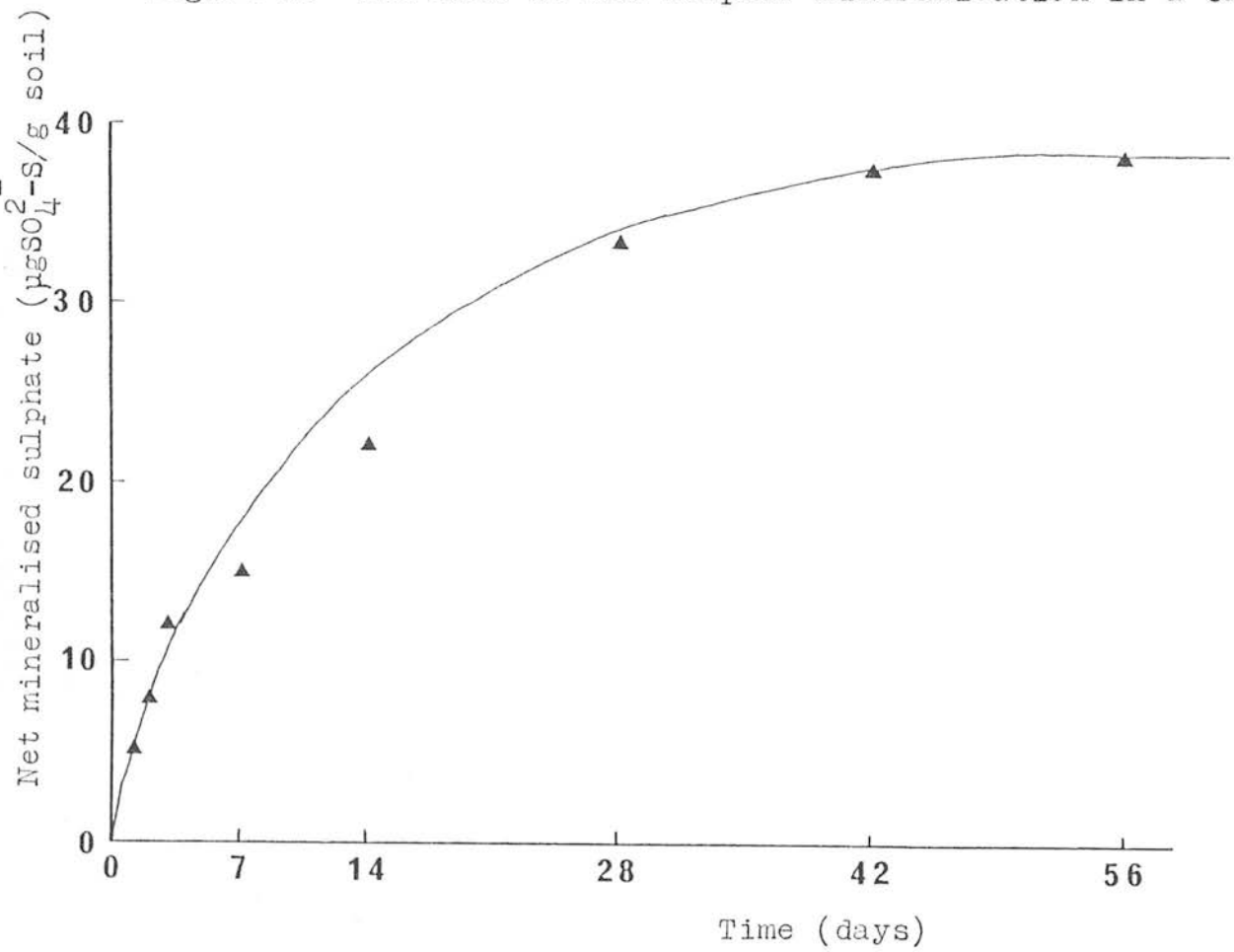


Figure 8. The rate of net sulphur mineralisation in a calcareous pelosol.



mineralisation. Other workers have also recognised this flush of mineralised sulphate (Williams, 1967; Kowalenko and Lowe, 1975ab) which is probably due to the Birch effect. The soil drying process gives rise to a source of readily utilised substrate (dead micro-organism tissue) which enables a high rate of micro-organism activity upon re-wetting of the soil. Also the microbial populations will be adjusting to the conditions imposed by the incubation procedure. After approximately 10 days these high rates began to fall off as the supply of easily metabolised substrate was exhausted. Both soils appeared to reach an equilibrium at about 35 days when mineralisation rates and immobilisation rates balanced, resulting in levels of sulphate which changed little with time. This equilibrium value for the Linhope soil was about 52μgSO_4^{2-}-S/g soil and 37μgSO_4^{2-}-S/g soil for the calcareous pelosol. Equilibrium would be expected since the flask is essentially a closed system (except for small losses as respired CO_2) but in the field, plant uptake of sulphate, leaching losses and the return of dead plant material to the soil combine to form a more dynamic and complex system. It is interesting to note that more sulphate was mineralised by the Linhope soil even though the C:S ratio was higher than that of the calcareous pelosol. Both soils contained similar amounts of total sulphur. However the Linhope soil contained much root and fresh plant material which could account for the greater quantities of sulphate mineralised.

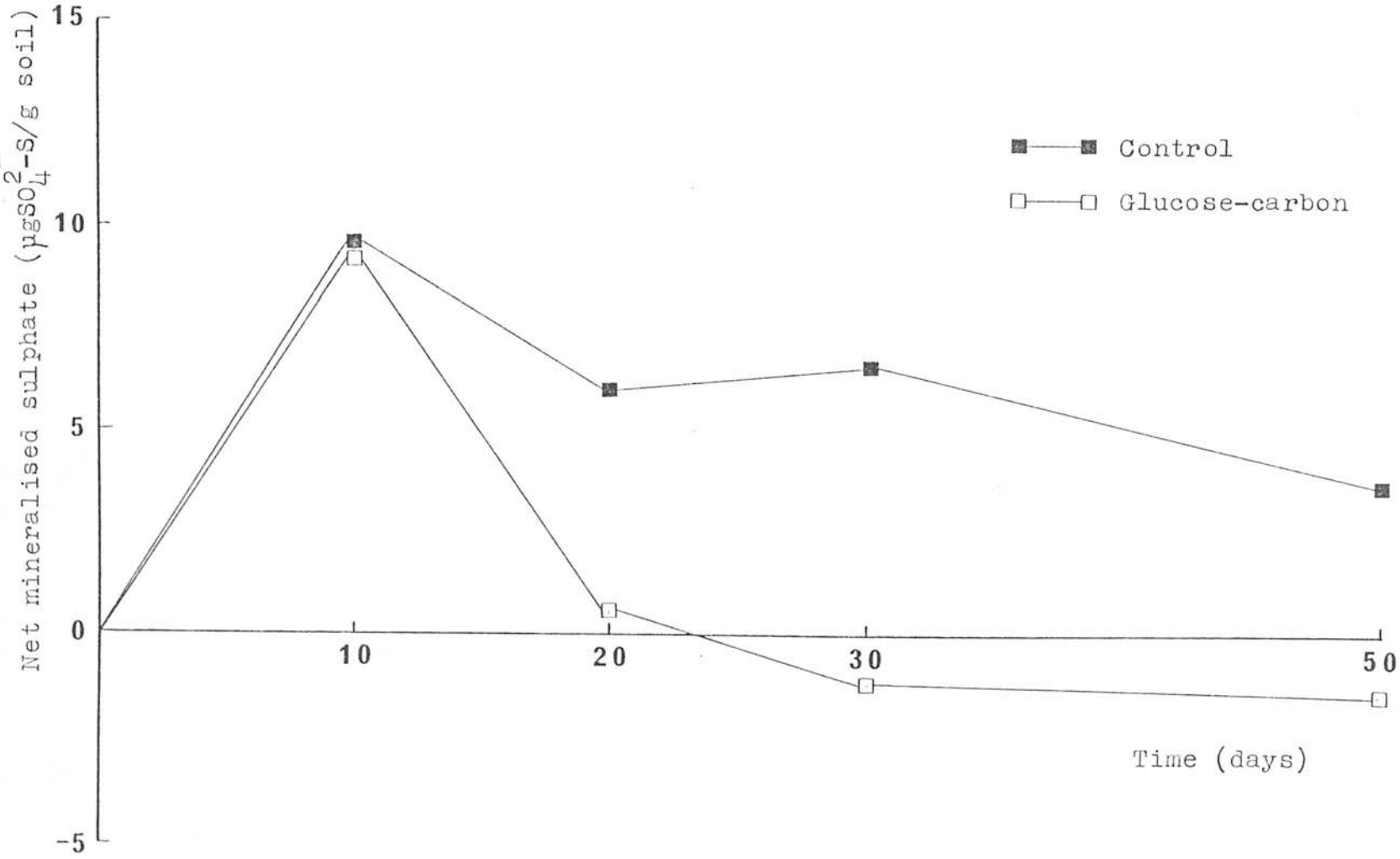
4.4.2 The effect of added glucose-carbon on the rate of net sulphur mineralisation

Four soils (Whitsome, Hobkirk, Linhope and Hexpath) were incubated at 30°C for 0, 10, 20, 30 and 50 days. The same soils were also incubated for similar lengths of time with added glucose-carbon (applied as glucose in solution at a rate of 1g glucose/20g soil). Figures 9, 10, 11 and 12 compare net sulphur mineralisation rates with and without added glucose-carbon for the four soils.

Added glucose-carbon (Fig. 9) greatly modified rates of net sulphur mineralisation in the Whitsome soils. As in the previous experiment a high initial rate of sulphate mineralised was observed and the added glucose-carbon did not appear to affect the flush of sulphate. After 10 days incubation the control showed a decrease in rate of net sulphur mineralisation and a steady state was established which was maintained for the duration of the experiment (the slight drop in the rate of net mineralisation between 30 and 50 days was probably insignificant). After the initial sulphate flush, where glucose-carbon had been added, there was a marked decrease in net mineralised sulphate and after approximately 20 days net immobilisation occurred.

In order to assimilate the added glucose-carbon the micro-organisms required sulphate in excess of that supplied by mineralisation. As a result the extractable soil sulphate fell below levels recorded at the beginning of the experiment since it became incorporated into

Figure 9. The effect of added glucose-carbon on the rate of net sulphur mineralisation in the Whitsome series soil.



microbial tissue. The effect of the glucose-carbon remained apparent for 50 days. Therefore the carbon was not completely removed from the system by respiration.

The Hobkirk soil showed distinctly similar trends to the Whitsome soil (Fig. 10) except that this soil mineralised larger quantities of sulphate such that the effect of added glucose-carbon did not cause net immobilisation. The initial sulphate flush was again evident but this time the added glucose-carbon reduced the effect slightly. An equilibrium sulphate concentration of approximately 14μgSO_4^{2-}-S/g soil was attained in the control after 20 days and remained constant until the end of the experiment. Where glucose-carbon was added a steady state was not reached and the amounts of sulphate mineralised were markedly less than for the control. In the Hobkirk soil the glucose-carbon addition did not widen the C:S ratio of the easily utilised substrate sufficiently to cause net immobilisation.

The Hexpath and Linhope soils (Figs. 11 and 12) showed very similar trends of sulphur mineralisation with time. The effect of added glucose-carbon was also very similar for both soils. Again the Linhope soil mineralised large quantities of sulphate initially ($40\mu gSO_4$-S/g soil) but after 20 days the balance between mineralisation and immobilisation changed, giving an equilibrium concentration of 25μgSO_4^{2-}-S/g soil. Similarly the Hexpath soil returned to an equilibrium concentration

Figure 10. The effect of added glucose-carbon on the rate of net sulphur mineralisation in the Hobkirk series soil.

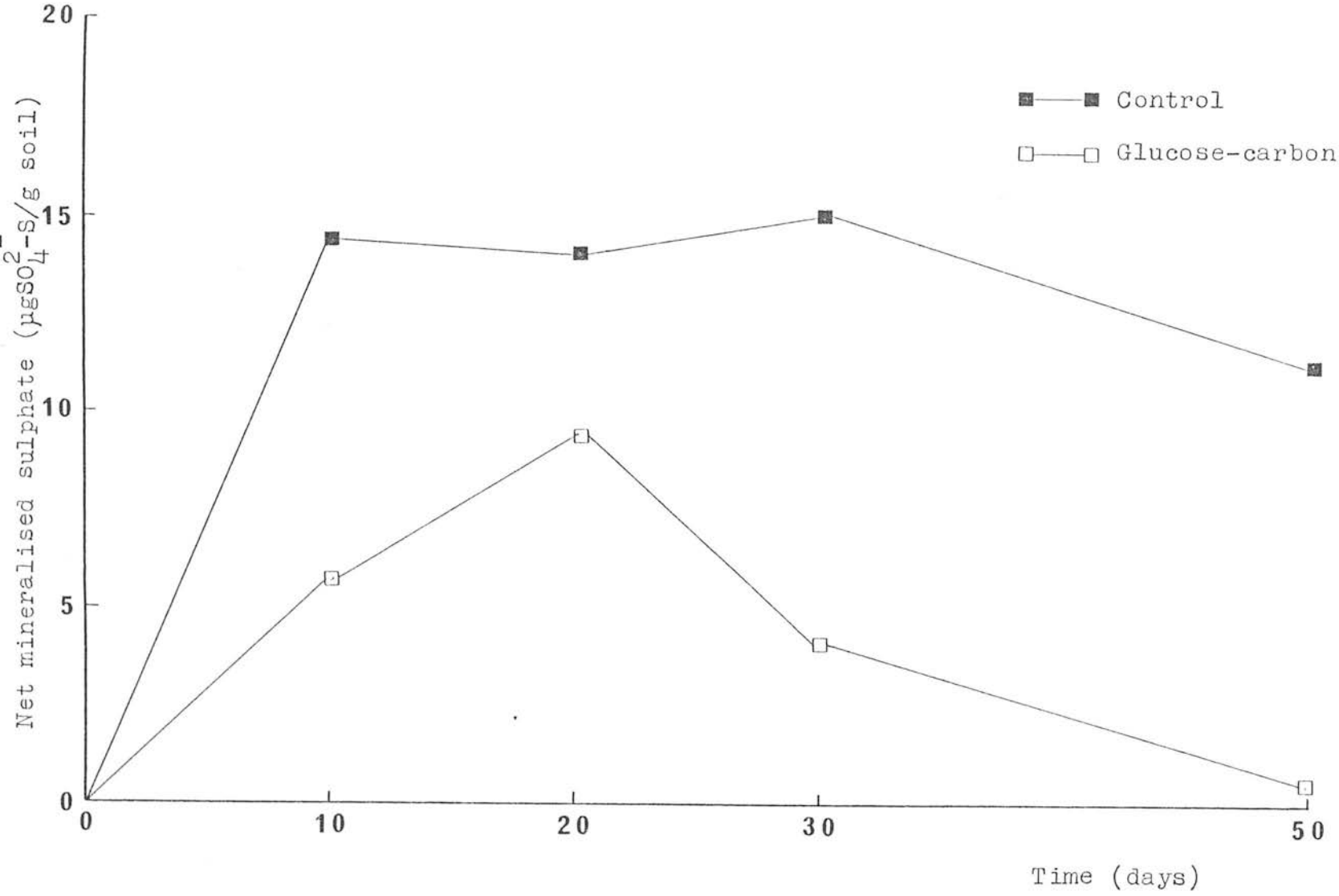


Figure 11. The effect of added glucose-carbon on the rate of net sulphur mineralisation in the Hexpath series soil.

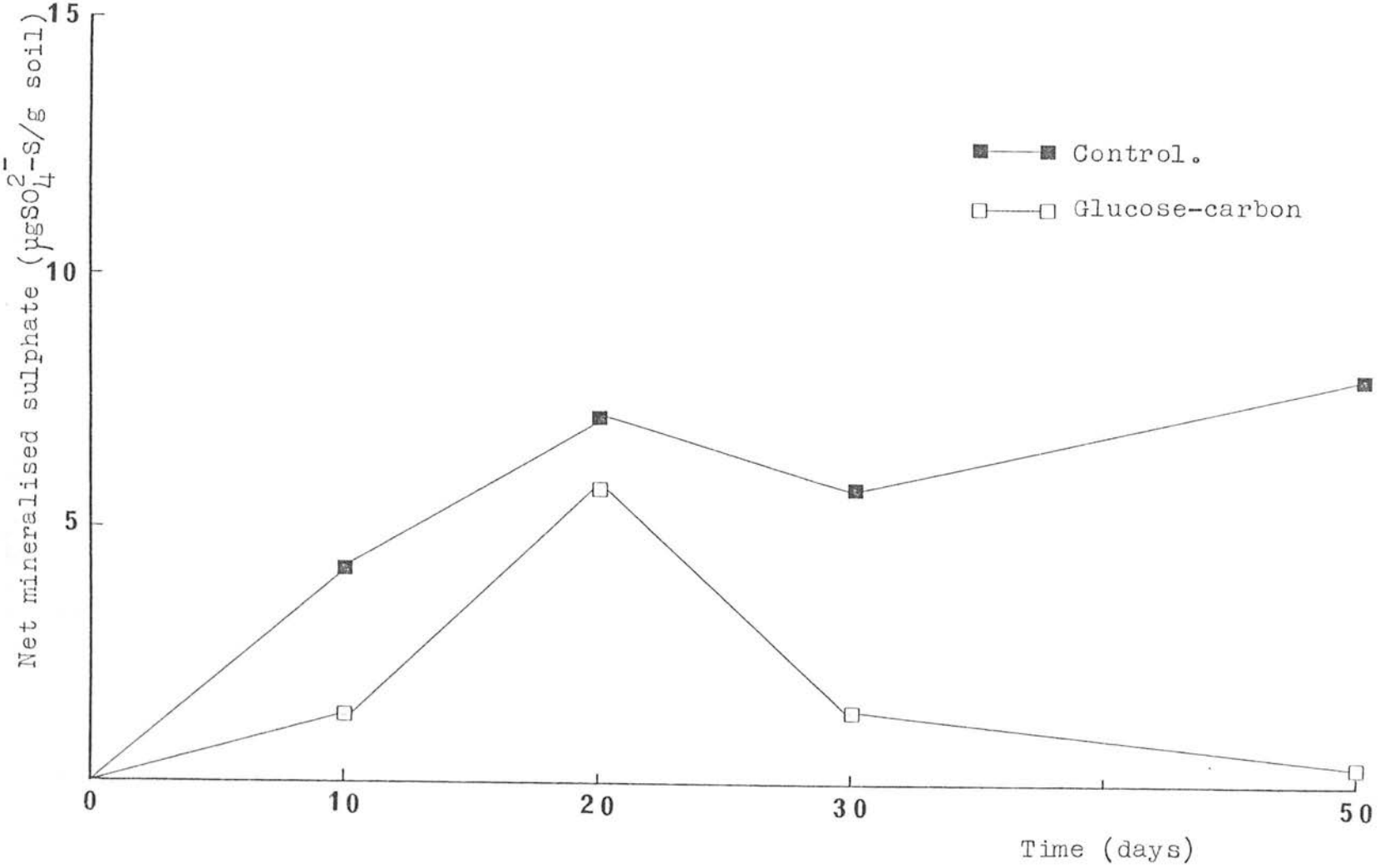
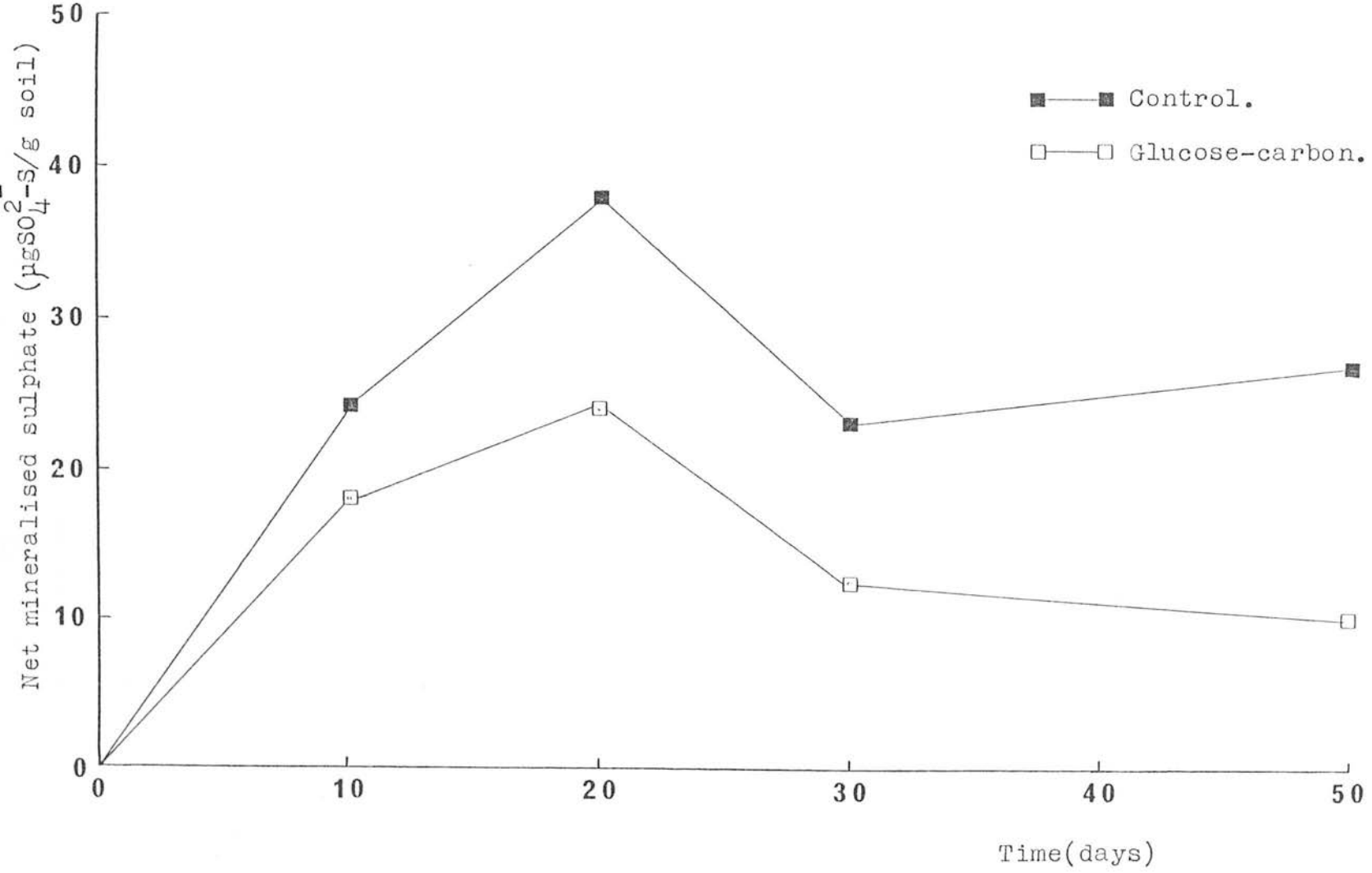


Figure 12. The effect of added glucose-carbon on the rate of net sulphur mineralisation in the Linhope series soil.



of about 7ugSO_4^{2-}-S/g soil after an initial flush of sulphate. For both soils the added glucose-carbon produced a similar pattern of sulphur mineralisation with time as was seen for the control except that sulphate values were lower. The theory that the micro-organisms required more sulphate in order to assimilate the added glucose-carbon explains the above differences between the control and added glucose-carbon treatments.

Although the four soils possessed a wide range of chemical properties a depression of sulphate levels mineralised was always observed when glucose-carbon was added. However only in the Whitsom soil did levels fall sufficiently to cause net immobilisation. Also all soils exhibited an initial flush of mineralised sulphate irrespective of glucose-carbon addition. Some soils attained an equilibrium concentration of mineralised sulphate within the 50 day incubation period. This probably represented the microfloral adjustment to the conditions imposed by the incubation.

4.4.3 The effect of temperature on net sulphur mineralisation.

Five soils (Whitsome, Hobkirk, Hexpath, Linhope and a calcareous pelosol) were incubated for 20 days at temperatures of 5, 10, 20 and 30°C. Figures 13 and 14 show net mineralised sulphate plotted against temperature.

It can be seen that all soils mineralised more sulphur when the temperature was increased from 5°C to 30°C. The range of temperatures employed was not wide

Figure 13. The effect of temperature on net sulphur mineralisation in the Hexham and Whitsome soil series and a calcareous pelosol

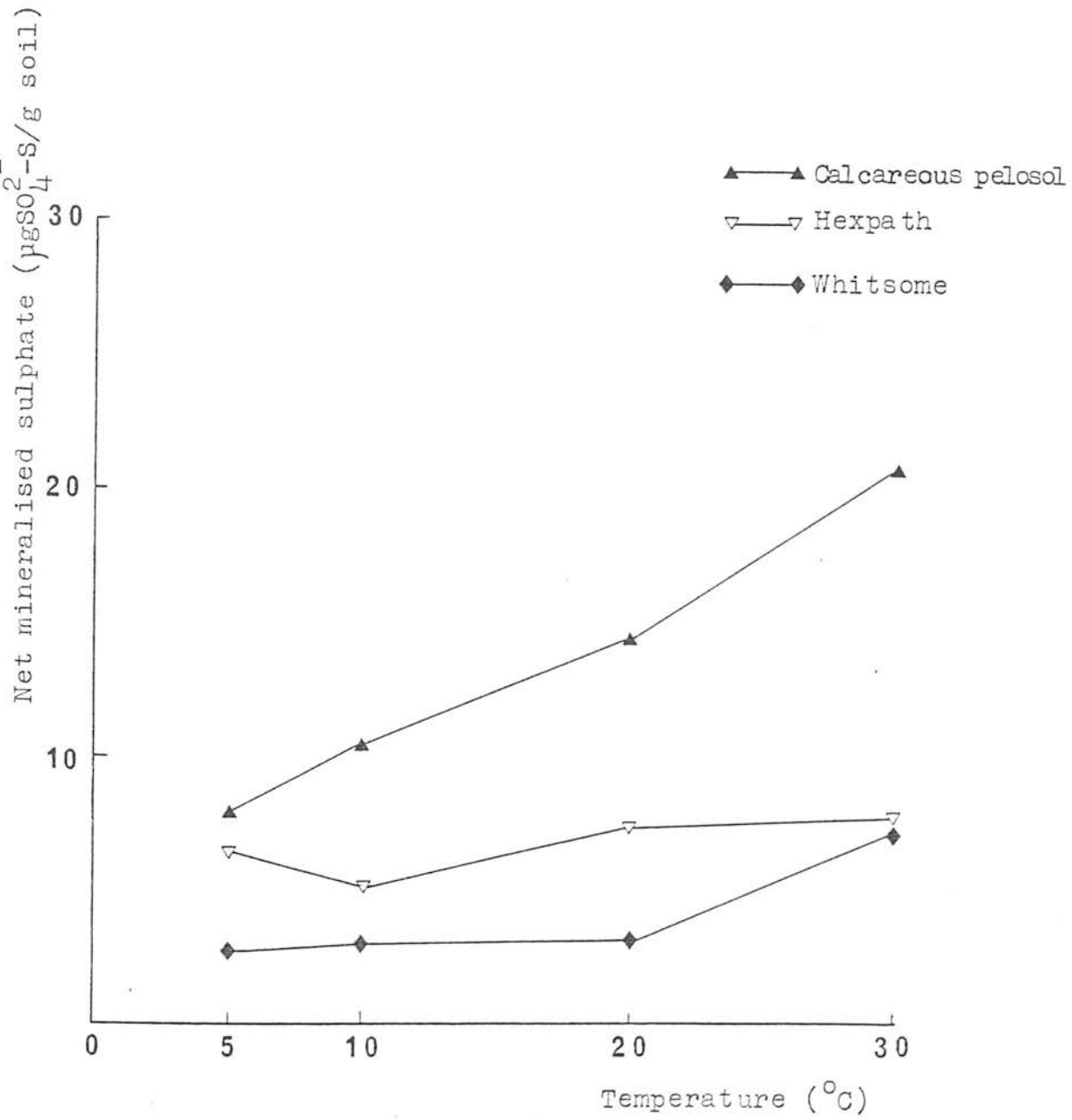
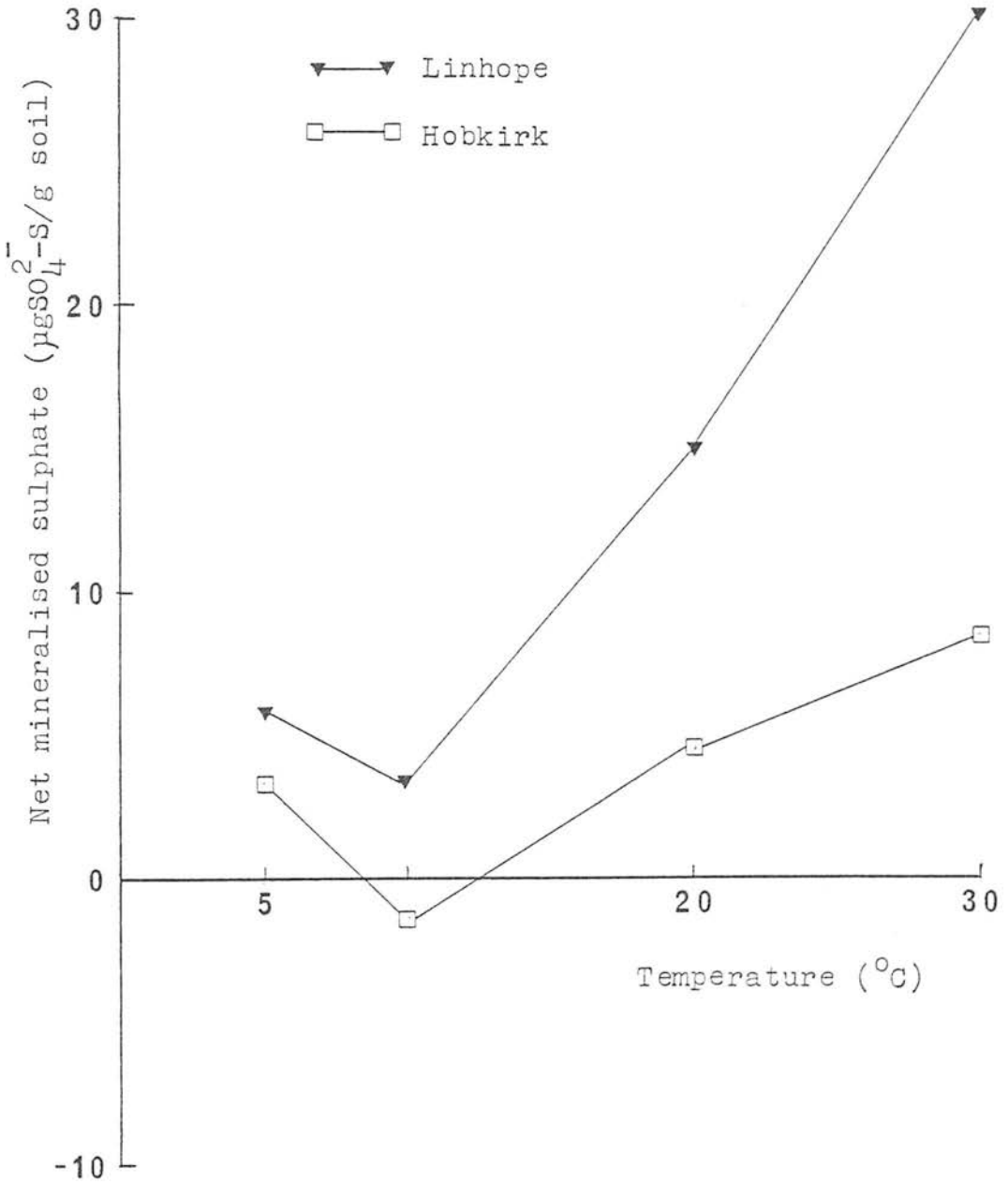


Figure 14. The effect of temperature on net sulphur mineralisation in the Hobkirk and Linhope soil series (20 day incubation).



enough to ascertain the optimum temperature for sulphur mineralisation but it was probably around 30°C. All soils showed some net mineralisation of sulphate at 5°C. However it is possible that not all the sulphate found resulted from mineralisation processes. During the 20 day incubation, chemical processes could have been operative (e.g. hydrolysis) in producing sulphate from organic sulphur. The fact that all soils showed net mineralisation at 5°C is of practical significance since the soils of Eastern Scotland would be at or below this temperature for much of the winter and early spring. In the Hobkirk, Linhope and Hexpath soils less sulphate appeared to be mineralised at 10°C than at 5°C. It is difficult to prove the significance of this observation but experimental error is the most likely explanation.

However increasing the temperature from 10°C to 20°C greatly increased amounts of sulphate mineralised in the Hobkirk, Linhope and calcareous pelosol. The Hexpath soil shows only slight increases in mineralised sulphate whilst the Whitsome soil shows no increase when the temperature is raised from 10°C to 20°C. The temperature rise from 20°C to 30°C caused the most marked increase in mineralised sulphate (Williams (1967) also found this relationship) for all soils except the Hexpath where only a small increase in mineralised sulphate was observed. It is well established that, up to an optimum value, increases in temperature produce increases in biological activity. Thus within the range 0-30°C the rate of

sulphur mineralisation would be expected to increase. However this would not necessarily lead to an increase in sulphate production since the rate of immobilisation would also increase. The temperature increase would therefore seem to favour the mineralisation type transformations especially in the Linhope and calcareous pelosol soils where considerable quantities of fresh plant material provides a source of readily-mineralisable sulphur. The cultivated Whitsome and Hobkirk soils contain less, easily-mineralised organic matter (and much less organic matter overall). Consequently the lower levels of microbial turnover will lessen the effect of temperature increase. The Hexpath soil has a high organic matter content (6.76%C) but this organic matter is probably resistant to sulphur mineralisation possibly due to the extremely wide C:S ratio of 136. In this case increased temperature did not greatly increase sulphur mineralisation over sulphate immobilisation.

4.4.4 The effect of added sulphate on net sulphur mineralisation.

The Linhope series soil was incubated at 30°C for 14 days with sulphur added as sodium sulphate in solution. The rates of sulphur applied were 0, 10, 20, 30, 40, 50 and 60µgS/g soil.

The sulphate additions did not affect the amount of sulphate mineralised during a 14 day incubation at 30°C (Table 46). The Linhope soil was used because of its high level of sulphur transformations but even when large

TABLE 46. The effect of added sulphate on the net sulphur mineralisation in the Linhope series soil.

Added sulphate ($\mu gS/g$ soil)	Mineralised sulphur ($\mu gSO_4^{2-}S/g$ soil)
0	12
10	13
20	11
30	11
40	11
50	13
60	11

TABLE 47. The effect of added nitrate on the net sulphur mineralisation in the Linhope series soil.

Added nitrate ($\mu gN/g$ soil)	Mineralised sulphur ($\mu gSO_4^{2-}S/g$ soil)
0	11
20	14
40	12
60	11
80	11
100	12
120	11

additions of sulphate were made (60µgS/g soil is equivalent to 150kgS/ha) no evidence of net immobilisation was seen.

4.4.5 The effect of added nitrate on net sulphur mineralisation.

The Linhope series soil was incubated at 30°C for 14 days with added nitrate applied as sodium nitrate in solution. The rates of nitrate applied were 0, 20, 40, 60, 80, 100 and 120µgN/g soil.

The nitrate additions did not affect the amounts of sulphate mineralised (Table 47) even though the nitrate was applied at rates three times greater than those commonly employed in the field. In order to assimilate the added nitrogen one might have expected net sulphate immobilisation. However a third element, namely carbon, could be limiting, such that the microflora is unable to utilise the added nitrogen. Future experiments should seek to examine the effect of added carbon, nitrogen and sulphate on the rate of net sulphate mineralisation. This work would determine whether the initial flush of sulphate masked any treatment effects and also help to assess the relative importance of C, N and S in controlling equilibrium soil sulphate concentrations.

4.4.6 The effects and interactions of added nitrate and sulphate on net sulphur mineralisation

The Linhope series soil was incubated at 30°C for 14 days with nitrate and sulphate (applied as sodium nitrate and sodium sulphate in solution) at the levels shown in Table 48.

TABLE 48. The effects and interaction of added nitrate and sulphate on the amount of sulphate mineralised by Linhope soil series

Added nitrate ($\mu g NO_3^-$-N/g soil)	Added sulphate ($\mu g SO_4^{2-}$-S/g soil)	Mineralised sulphate ($\mu g SO_4^{2-}$-S/g soil)
0	0	11
10	0	8
20	0	11
30	0	10
40	0	9
50	0	10
0	20	10
10	20	9
20	20	10
30	20	8
40	20	11
50	20	8

Again no effect of added sulphate or nitrate on amounts of sulphate mineralised was observed (Table 48). Similarly no interaction of sulphate and nitrate was observed either. As mentioned previously a three way interaction experiment investigating C, N and S effects upon net sulphur mineralisation would aid understanding of the mineralisation process.

4.4.7 Incubation Experiment I. A comparison of the ability of several soils to mineralise sulphur

The soils described in Tables 1, 2, 3 and 4 were incubated at 30°C for 14 days. Levels of net mineralised sulphate were recorded and correlated with the following soil chemical properties:- total carbon, total sulphur, C:S ratio, extractable sulphate, HI-reducible sulphur and carbon-bonded sulphur. The results appear in Table 49, ranked according to the amount of sulphate mineralised by each soil.

The correlation matrix (Table 51) shows that the amount of sulphate mineralised by a soil is apparently significantly correlated with all the following fractions of soil sulphur; total sulphur, carbon-bonded sulphur, HI-reducible sulphur and extractable sulphate. However inter-correlation between the above parameters makes it difficult to obtain direct causal relationships with any one parameter. The amount of sulphate mineralised by a soil correlates better with HI-reducible sulphur than carbon-bonded sulphur which indicates that the former

TABLE 49. A list of soils showing values of net mineralised sulphate and chemical parameters

Soil Type	Mineralised sulphate* (μgSO_4^{2-}-S/g soil)	Total carbon (%C)	C:S ratio	Total S (μgS/g soil)	HI reducible S (μgS/g soil)	Extractable sulphate (μgSO_4^{2-}-S/g soil)	C bonded S (μgS/g soil)
Kilmarnock p.p.	24.4	5.04	64	788	237	20.9	551
Stirling	21.0	2.12	25	862	546	179.0	316
Ragdale p.p.	20.2	5.21	62	838	285	38.8	453
Calcareous pelosol	16.8	5.27	45	1175	498	5.0	677
Tedburn	14.7	3.69	58	635	282	5.0	353
Yellowland	12.3	4.43	59	755	411	12.0	344
Non Calc. pelosol	11.0	3.01	52	580	276	2.3	304
Dunsford	10.9	3.81	60	635	289	4.5	346
Kilmarnock a.	10.8	2.38	72	330	148	12.0	182
Neath	10.7	3.12	57	555	234	9.0	321
Denchworth	10.4	3.96	53	749	340	19.6	409
Linhope	10.0	7.90	77	1025	468	23.0	557
Halstow	9.8	2.77	52	535	234	6.0	301
Whitsome (B.A.M.)/	9.5	1.98	49	405	170	7.0	235
Humbie p.p.	9.3	3.24	72	450	212	15.2	238
Ragdale a.	9.1	1.45	57	255	131	15.9	124
Humbie a.	8.3	2.20	60	365	122	8.4	243
Eckford	8.2	4.54	133	410	160	5.5	250
Sourhope	8.1	3.82	68	560	234	21.4	326
Crichton	7.9	3.12	69	455	186	4.9	269

*Sulphate mineralised during 14 days incubation at 30°C

/BAM = Blackaddermount Farm.

a = arable

p.p. = permanent pasture.

TABLE 49. (contd.) A list of soils showing values of net mineralised sulphate and other chemical parameters

Soil Type	Mineralised sulphate* (μgSO_4^{2-}-S/g soil)	Total carbon (%C)	C:S ratio	Total S (μgS/g soil)	HI reducible S (μgS/g soil)	Extractable sulphate (μgSO_4^{2-}-S/g soil)	C bonded S (μgS/g soil)
Macmerry	7.8	3.08	58	535	157	6.6	378
Biel	7.8	2.83	63	450	183	17.2	267
Highweek	7.1	2.53	50	510	228	7.3	282
Easter Bush	7.0	2.46	52	475	231	5.0	244
Hobkirk	7.0	3.78	98	380	125	6.4	255
Neath p.p.	6.8	4.07	72	565	218	6.5	347
Pow	6.8	1.95	48	405	160	19.6	245
Dreghorn	6.0	2.19	63	347	128	7.8	219
Boyndie	5.8	1.79	77	233	99	21.4	134
Trusham	5.5	2.19	42	520	209	2.0	311
Hexpath	5.4	6.76	136	498	218	6.5	280
Wix	5.3	1.08	54	200	87	3.2	113
Woodhead Farm	5.3	2.93	72	405	160	7.1	245
Darvel	5.2	2.06	68	303	99	10.6	204
Kedslie	5.2	2.93	68	429	154	3.8	275
Whitsome (D.G.H.)†	4.1	2.27	48	475	190	5.0	285
Holsworthy	4.0	3.42	63	545	228	10.3	317
Moorgate	3.7	4.99	93	535	163	8.3	372
Cessford	3.0	4.14	68	606	276	8.0	330
Winton sub-soil	2.9	0.64	43	150	51	12.0	99

*Sulphate mineralised during 14 days incubation at 30°C.

†D.G.H. = Dykegatehead Farm
p.p. = permanent pasture

fraction is more reactive and a likely precursor of mineralised sulphate-sulphur. Extractable soil sulphate correlates well with mineralised sulphate because the extractable sulphate represents a soils ability to provide (mineralise) sulphate in the face of plant uptake and leaching losses. Of all the measures of soil sulphur tested total sulphur correlated best with mineralised sulphate. Therefore although total sulphur measurements do not specify the chemical nature of the soil sulphur they provide the best indication of amounts of mineralisable sulphur present in the soil.

The carbon; sulphur ratio was noticeably poorly correlated with amounts of mineralised sulphate. Some workers have suggested that carbon; sulphur ratios are important in determining amounts of sulphur mineralised (Kowalenko and Lowe, 1975) whilst other workers doubt the importance of this ratio (Swift, 1977). Several other significant correlations appear in the correlation matrix and most of these emphasise that the sulphur of British soils is almost entirely organic in nature. For example percent carbon showed significant correlations with total sulphur, carbon-bonded sulphur and HI-reducible sulphur. Several other of the significant correlations present must be treated with caution since several variables are not independent of each other, for example the percentage of total S occurring as HI-reducible sulphur is highly correlated with the percentage of total sulphur occurring as carbon-bonded sulphur.

Conversion of the parameters into percentages of the total sulphur (Table 50) did not improve the correlations and only the percentage of the total sulphur occurring as extractable sulphate correlated with the percentage of the total sulphur mineralised.

In an attempt to find a more exact relationship between mineralised sulphate and the other soil properties measured, multiple regressions/correlations were examined. This entailed using the computer to calculate multiple correlation coefficients between mineralised sulphate and various combinations of the other soil chemical properties. The variables which contributed least to the correlation were automatically discarded in the calculation. A combination of total sulphur and extractable soil sulphur provided the only multiple correlation coefficient significant at the 5 percent level of significance. The correlation obtained was slightly better than for total sulphur alone. The regression equation was,

$$\text{mineralised sulphate} = 0.013 \text{ extractable sulphate} + 0.057 \text{ total sulphur} + 1.476.$$

Such an equation provides a means of predicting the sulphur supplying power of a soil from knowledge of the total sulphur and extractable soil sulphate - two easily determined values. Whilst this indirect approach is not the most desirable it would seem the only one available until labile organic sulphur compounds can be identified and measured.

TABLE 50. A list of soils showing values of net mineralised sulphate and other chemical parameters (expressed as a percentage of the total soil sulphur)

Soil Type	% of total S mineralised*	HI reducible S as % of total S	Extractable SO_4^{2-} as % of total S	C bonded S as % of total S
Kilmarnock p.p.	3.10	30.1	2.65	69.9
Stirling	2.44	63.3	20.77	36.7
Ragdale p.p.	2.41	34.0	4.63	64.0
Calcareous pelosol	1.43	42.4	0.43	57.6
Tedburn	2.31	44.4	0.79	55.6
Yellowland	1.63	54.4	1.59	45.6
Non Calc. pelosol	1.90	47.6	0.40	52.4
Dunsford	1.72	45.0	0.71	55.0
Kilmarnock a	3.27	44.8	3.64	55.2
Neath	1.93	42.2	1.62	57.8
Denchworth	1.39	45.4	2.62	54.6
Linhope	0.98	45.7	2.24	54.3
Halstow	1.83	43.7	1.12	56.3
Whitsome (B.A.M.)/	2.35	42.0	1.73	58.0
Humbie p.p.	2.07	47.1	3.38	52.9
Ragdale a	3.57	51.4	7.07	48.6
Humbie a	2.27	33.4	2.30	66.6
Eckford	2.00	39.0	1.34	61.0
Sourhope	1.45	41.8	3.82	58.2
Crichton	1.74	40.9	1.08	59.1

*mineralised during 14 days incubation at 30°C.

/B.A.M. = Blackaddermount Farm.
p.p. = permanent pasture
a = arable

TABLE 50 (contd.) A list of soils showing values of net mineralised sulphate and other chemical parameters (expressed as a percentage of the total soil sulphur)

Soil Type	% of total S mineralised*	HI reducible S as % of total S	Extractable SO_4^{2-} as % of total S	C bonded S as % of total S
Macmerry	1.46	29.3	1.23	70.7
Biel	1.73	40.7	3.82	59.3
Highweek	1.39	44.7	1.43	55.3
Easter Bush	1.47	48.6	1.05	51.4
Hobkirk	1.84	32.9	1.68	67.1
Neath p.p.	1.20	38.6	1.15	61.4
Pow	1.68	39.5	4.84	60.5
Dreghorn	1.73	36.9	2.25	63.1
Boyndie	2.49	42.5	9.18	57.5
Trusham	1.06	40.2	0.38	59.8
Hexpath	1.08	43.8	1.31	56.2
Wise	2.65	43.5	1.60	56.5
Woodhead Farm	1.31	39.5	1.75	60.5
Darvel	1.72	32.7	3.50	67.3
Kedslie	1.21	35.9	0.89	64.1
Whitsome (D.G.H.)/	0.86	40.0	1.05	60.0
Holsworthy	0.73	41.8	1.89	58.2
Moorgate	0.69	30.5	1.55	69.5
Cessford	0.50	45.5	1.32	54.5
Winton sub-soil	1.93	34.0	8.00	66.0

*Mineralised during 14 days incubation at 30°C.

/D.G.H. = Dykegatehead Farm.
p.p. = permanent pasture

TABLE 51. A correlation matrix showing the relationship between mineralised sulphate and some soil chemical parameters

	Mineralised sulphate (μgS/g soil)	Total carbon (%C)	C:S ratio	Total S (μgS/g soil)	HI-reducible S (μgS/g soil)	Extractable sulphate (μgS/g soil)	Carbon bonded S (μgS/g soil)
Total carbon (%C)	0.303						
C:S ratio	- 0.252	0.527***					
Total S (μgS/g soil)	0.653***	0.708***	- 0.163				
HI reducible S (μgS/g soil)	0.606***	0.546***	- 0.255	0.919***			
Extractable sulphate (μgS/g soil)	0.497***	- 0.055	- 0.297	0.312*	0.505***		
Carbon bonded S (μgS/g soil)	0.576***	0.756***	- 0.056	0.937***	0.731***	0.079	
% of total S mineralised	0.515***	- 0.359*	- 0.143	- 0.242	- 0.182	0.226	- 0.294
HI reducible S as % of total S	0.226	- 0.048	- 0.277	0.251	0.589***	0.488**	- 0.068
Extractable SO_4^{2-} as % of total S	0.308*	- 0.300	- 0.282	- 0.011	0.203	0.890***	- 0.223
Carbon bonded S as % of total S	- 0.246	0.038	0.280	- 0.264	- 0.598***	0.499***	0.059

	% of total S mineralised	HI reducible S as % of total S	Extractable sulphate as % of total S
	0.139		
	0.393***	0.408**	
	- 0.147	- 0.999***	- 0.415**

4.5 Sulphur-35 Experiments

Whilst the previous section (incubation experiments) expounded some of the factors affecting sulphur mineralisation, no information on the transformations involved in the conversion of organic sulphur to sulphate was obtained. Hence the sulphur-35 work was designed to increase knowledge of the relative rates of mineralisation/immobilisation and the chemical forms of the recently incorporated sulphur. An attempt was also made to identify a labile organic sulphur fraction (that which is especially susceptible to mineralisation). Identification of such a fraction would allow soil sulphur extractants to be assessed for their ability to predict the longer term sulphur supplying power of soils.

4.5.1 Experiment I. Incubation of Whitsome series soil with sulphur-35.

The soil was incubated with sulphur-35 labelled sulphate for 75 days, with and without added glucose-carbon, as described in section 3.7.1. After incubation the soil was immediately air dried to 'fix' the labelled sulphur in the various soil sulphur fractions. The amount of sulphur-35 incorporated into organic forms was measured by extracting the soil with monocalcium phosphate solution. The degree of incorporation can be calculated by subtracting the extracted label from the amount of label originally added (preliminary adsorption, experiments showed conclusively that Whitsome soil does not adsorb sulphate).

However it is probable that the extracting solution will dissolve some simple sulphur-35 labelled organic compounds and therefore the enhanced activity of the extract will lead to underestimated levels of incorporation. Practical difficulties incurred when attempting to separate labelled sulphate from labelled water soluble organic compounds are discussed below (section 4.6.6). The decay of sulphur-35 was corrected to the date of labelled sulphate addition. Incubation for 75 days in the absence of added glucose-carbon resulted in incorporation of 28 percent of the sulphur-35 originally applied. Where glucose-carbon was added to the soil, incorporation of sulphur-35 into organic forms increased to 49 percent of that originally applied.

Therefore it appears that the soil microflora has immobilised more labelled sulphate in order to assimilate the added glucose-carbon. To further check the validity of this type of experiment the total recovery of applied label can be calculated by adding the amount of extracted label to the amount of residual label remaining in the extracted soil and comparing this with the amount originally added.

Similar work (Freney et al., 1971) showed a low recovery of 80-89 percent of the applied sulphur-35. However work here gave a recovery of 99 percent of the applied label where glucose-carbon was added and 97.5 percent where glucose-carbon was omitted. Taking into account experimental error no significant loss of

TABLE 52. The levels of sulphur-32 in the soil sulphur fractions before and after incubation (Whitsome series).

Soil sulphur fraction	Initial sulphur levels (µgS/g)	Final sulphur levels (+ carbon) (µgS/g)	Final sulphur levels (control) (µgS/g)
Extractable sulphate	20*	21	23
HI-reducible sulphur	90	115	93
Carbon-bonded sulphur	210	200	217
Total sulphur	300	315	310

*this value includes the 15ugS/g added as a carrier.

TABLE 53. The distribution of sulphur-35/ between the soil sulphur fractions after incubation (Whitsome series).

Soil sulphur fraction	Amount of sulphur-35 (+ carbon) (mCi S-35)	Amount of sulphur-35 (control) (mCi S-35)
Extractable sulphate	1.02	1.41
HI-reducible sulphur	0.66	0.48
Carbon-bonded sulphur	0.30	0.06
Total sulphur	0.96	0.54

/2mCi of sulphur-35 was originally added.

sulphur-35 occurred during the incubation (for example, by volatilisation). Table 53 shows the amounts of sulphur-35 which have been incorporated into the various soil sulphur fractions. The HI-reducible sulphur-35 included only organic sulphur forms because the determination was performed on a soil sample previously leached with monocalcium phosphate solution. This procedure was adopted because the large amount of sulphur-35 remaining as sulphate would confuse discussion of the redistribution of sulphur-35 after incubation. The carbon-bonded sulphur was determined as the difference between total sulphur and HI-reducible sulphur since direct methods are considered unreliable. (Freney et al., 1970). The soil incubated without glucose-carbon contained 89 percent of the incorporated sulphur-35 as HI-reducible sulphur and 11 percent as carbon-bonded sulphur. The soil incubated with added glucose-carbon shows a different distribution of incorporated label between the two sulphur forms, with 69 percent in the HI-reducible pool and 31 percent in the carbon-bonded pool. While both treatments showed greater incorporation into the HI-reducible pool, the added glucose-carbon enhanced incorporation into the carbon-bonded sulphur fraction. Such a difference due to the effect of added glucose-carbon was not observed by Freney et al., (1971).

The incubation period markedly reduced the specific activity of the extractable soil sulphate (Table 54) in

both the control and added glucose-carbon treatments. Immobilisation could not have caused this drop in specific activity since sulphur-32 and sulphur-35 would be incorporated into the micro-organisms at a similar rate. Therefore this dilution of the extractable sulphur-35 is due to the mineralisation of indigenous sulphur-32 from the organic soil sulphur pool. The added carbon caused a greater reduction in specific activity of the extractable sulphur indicating that carbon increases the turnover of indigenous sulphur-32 in addition to turnover of sulphur-35. It is clear that both sulphur-32 and sulphur-35, incorporated into soil organic molecules, is subsequently re-mineralised to sulphate but the relative susceptibilities of the labelled and unlabelled sulphur cannot be deduced from this particular experiment.

The levels of sulphur-32 in the various soil sulphur fractions before and after incubation are shown in Table 52. Incubation in the absence of added glucose-carbon has not substantially altered the distribution of sulphur-32 between the soil sulphur fractions except that net mineralisation of 3µg sulphur-32/g soil has occurred. Where carbon was added results suggest that some conversion of carbon-bonded sulphur into HI-reducible sulphur has occurred (a phenomenon noticed by Freney et al., 1971) and only 1µg sulphur-32/g soil was produced by net mineralisation. These values of net mineralisation are very interesting when one considers that large amounts

TABLE 54. The specific activity of the soil sulphur fractions (Whitsome series)

Soil sulphur fraction	Specific activity (+ carbon treatment) (μCi/mgmS)	Specific activity (control) (μCi/mgmS)
Extractable sulphate	97.1*	122.6
HI-reducible sulphur	11.5	10.3
Carbon-bonded sulphur	3.0	0.6
Total sulphur	6.4	3.5

*The initial specific activity of the extractable sulphate was 200μCi/mgmS.

of sulphur-32 and the associated label must have been immobilised to account for the percentages of incorporated sulphur-35 noted above. Since the percentages of incorporated sulphur-35 can be obtained from Table 53 one can assume that at least a similar proportion of the sulphate-32 initially present would also become incorporated into organic forms. By this assumption soil incubated without added carbon resulted in the immobilisation of 5.6μgSO_4^{2-}-S/g which requires gross mineralisation of 8.6μgSO_4^{2-}-S/g to give a net mineralisation value of 3μgSO_4^{2-}-S/g soil. Similarly, for the case where glucose-carbon is added, 9.8μgSO_4^{2-}-S/g was immobilised and 10.8μgSO_4^{2-}-S/g soil must have been mineralised to give net mineralisation of 1μgSO_4^{2-}-S/g soil. All the above values of immobilisation and gross mineralisation are not entirely accurate because the sulphur-32 and associated label have probably undergone many mineralisation/immobilisation cycles during the 75 days incubation. Therefore values quoted above are undoubtedly underestimates. However more information about the relative rates of immobilisation and mineralisation is revealed than could be obtained from net mineralisation values alone. The gross mineralisation values show that added carbon has increased the turnover of soil sulphur but this is not made evident by referring to net mineralisation values. The findings are in agreement with those of other workers (Freney et al., 1971; Goh and Tsuji, 1979) who state that mineralisation

and immobilisation processes occur simultaneously in the soil.

4.5.2 Experiment II. Incubation of Stirling series soil with sulphur-35.

The Stirling soil was incubated with sulphur-35 and added glucose-carbon for 75 days. Soil sub-samples were taken at intervals to follow the incorporation of S-35 into the various soil sulphur fractions. Details of experimental techniques and incubation conditions are found in section 3.7.2.

Results show (Table 55) that the levels of sulphur-32, in the four soil sulphur fractions investigated, remain relatively unaltered throughout the incubation. However, the extractable soil sulphate levels do fall significantly indicating net sulphate immobilisation (especially noticeable after the first ten days). The incubation conditions imposed on the soil have therefore not markedly affected the distribution of indigenous soil sulphur between the sulphur fractions.

The soil was immediately air dried after each sampling to 'fix' the labelled sulphur in the various soil sulphur fractions. The activity of the extractable soil sulphur was determined to enable quantification of the sulphur-35 incorporation into organic forms. The assumptions made in the calculation of percentage incorporation have been discussed in section 4.5.1. Figure 15 shows that 35 percent of the added sulphur-35 is incorporated (immobilised) into insoluble organic

TABLE 55. The levels of sulphur-32 in the Stirling soil sulphur fractions, before and during incubation

Soil sulphur fraction	Initial sulphur levels (µgS/g)	S levels after 10 days incub. (µgS/g)	S levels after 25 days incub. (µgS/g)	S levels after 50 days incub. (µgS/g)	S levels after 75 days incub. (µgS/g)
Extractable sulphate	26.8*	24.5	23.4	23.7	23.5
HI-reducible sulphur	200	192	212	215	205
Carbon-bonded sulphur	225	228	223	202	205
Total sulphur	425	420	435	417	410

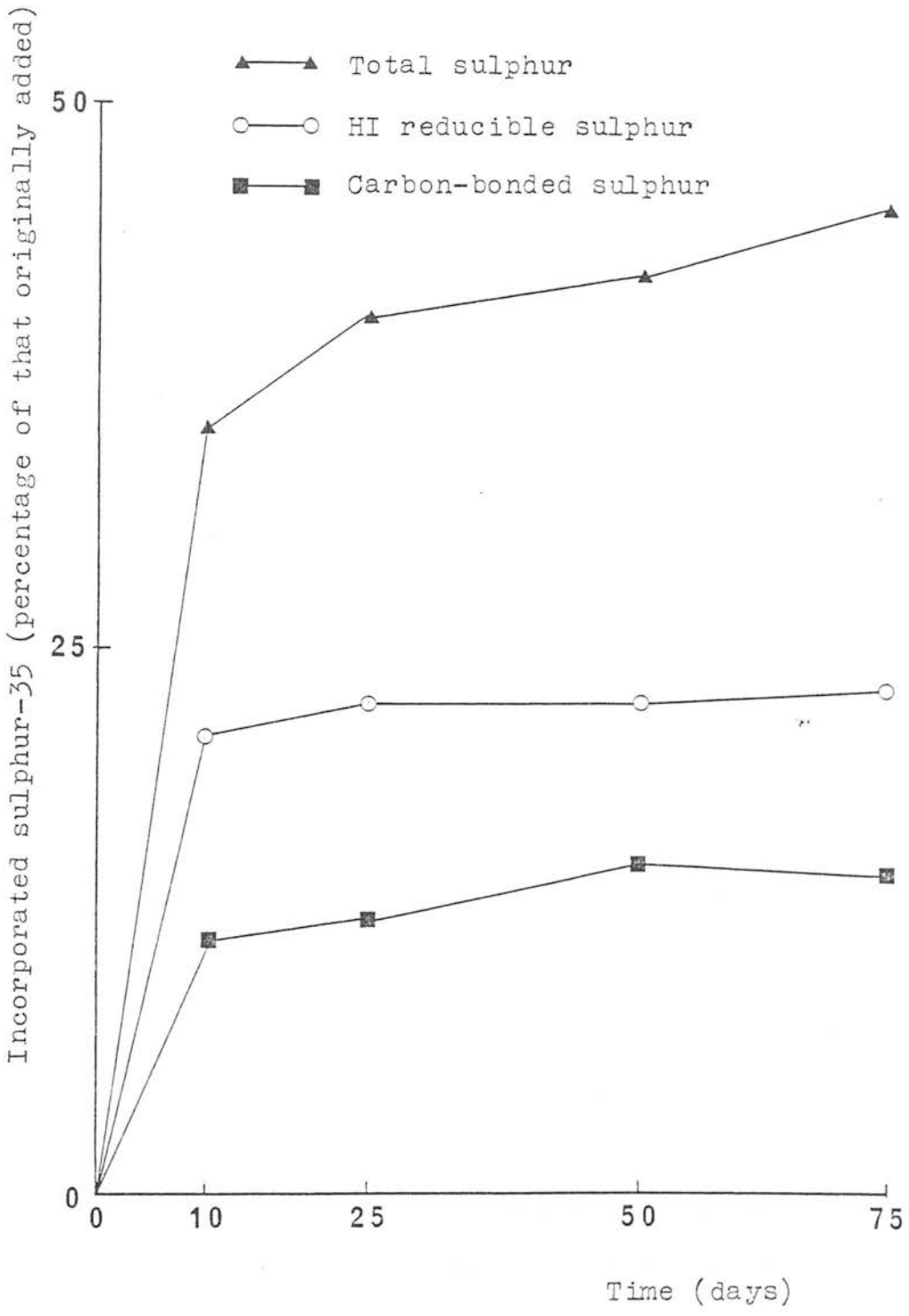
*this value includes 5ugS/g added as a carrier

TABLE 56. The distribution of sulphur-35/ between the Stirling soil sulphur fractions during incubation

Soil sulphur fraction	Amount of sulphur-35 (mCi)			
	10 days incubation	25 days incubation	50 days incubation	75 days incubation
Extractable sulphate	3.25	2.91	2.67	2.49
HI-reducible sulphur	1.09	1.24	1.27	1.28
Carbon-bonded sulphur	0.64	0.75	0.92	0.97
Total sulphur	1.73	1.99	2.19	2.25

/5mCi of sulphur-35 was originally added.

Figure 15. The incorporation of sulphur-35 into Stirling soil series organic sulphur fractions during the incubation period



sulphur after just 10 days of incubation. Over the next 65 days only a further 10 percent of the applied sulphur-35 becomes incorporated. Figure 15 only indicates rate of net sulphur-35 incorporation. Therefore the high initial rate of sulphur-35 incorporation, evident in the figure, is because remineralisation of the incorporated sulphur-35 is not significant at this early stage of incubation. (It is possible that gross sulphur-35 incorporation proceeds at a constant rate throughout the incubation.)

Since the percentages of incorporated sulphur-35 increased at each sampling date no true equilibrium between organic sulphur-35 and sulphate-35 had been reached. However the increases at 25, 50 and 75 days are small and it is interesting to note that near equilibrium is attained within 25 days which indicates the high turnover of sulphur in the Stirling soil.

The partitioning of the incorporated sulphur-35 between carbon-bonded and HI-reducible sulphur is similar to that observed for the Whitsome soil. Again, more of the sulphur-35 is found in the HI-reducible form, although 43 percent of the incorporated sulphur-35 occurs as carbon-bonded sulphur (compared to 31 percent for the Whitsome soil incubated with added glucose-carbon). There is very little change in the amount of sulphur-35 incorporated as HI-reducible sulphur after 25 days while the carbon-bonded S-35 does show a gradual increase with time. This is an indication of the more transient nature of the HI-

reducible fraction and the reserve nature of the carbon-bonded sulphur pool which takes more time to equilibrate with the added sulphur-35. Since levels of sulphur-32 remain almost unchanged by incubation the specific activity data (Table 57) closely resembles the changes in amounts of sulphur-35 reported in Table 56. The specific activity of the extractable sulphur falls drastically during the first 10 days as the unincorporated sulphur-35 is diluted by mineralised sulphur-32. After the first 10 days the proportion of sulphur-35 to sulphur 32 in the soil extractable sulphur remains fairly constant although true equilibrium between the sulphur-32 and the sulphur-35 of the organic matter and extractable sulphur was not attained. Such an equilibrium would probably take a long time, if ever, to become established. The specific activities of the carbon-bonded and HI-reducible sulphur, after 10 days incubation, illustrated how quickly the sulphur-35 was distributed through the soil sulphur fractions. Small increases in specific activity at subsequent sampling dates indicated that while near equilibrium was soon attained complete equilibrium was not reached within 75 days of incubation.

As mentioned above an estimate of gross sulphate mineralisation can be obtained from values of percentage sulphur-35 incorporation, amounts of associated sulphate-32 and the net mineralisation or immobilisation of sulphate-32. Percentage incorporation data suggests

TABLE 57. The specific activity of the Stirling soil sulphur fractions

Soil sulphur fraction	Specific activity (μCi/mgmS) 10 days incubation	25 days incubation	50 days incubation	75 days incubation
Extractable sulphate	132.7*	124.4	112.7	106.0
HI-reducible sulphur	5.68	5.91	5.85	6.24
Carbon-bonded sulphur	2.81	3.36	4.55	4.73
Total sulphur	4.12	4.57	5.25	5.49

*The initial specific activity of the extractable sulphate was 186.6 μCi/mgmS.

TABLE 58. The percentages of the Stirling and Whitsome soil sulphur fractions actively undergoing transformation*

Soil sulphur fraction	Stirling soil (+ glucose-C)	Whitsome soil (control)	Whitsome soil (+ glucose-C)
HI-reducible sulphur	5.9	8.0	12.0
Carbon-bonded sulphur	4.5	0.5	3.0
Total sulphur	5.2	3.0	6.0

* (based on the specific activity of the extractable sulphate)

that, after 10 days incubation, 9.4µgS/g has been incorporated while extractable sulphate-32 levels indicated a net immobilisation of 2.3µgS/g. Therefore gross mineralisation in the first 10 days produced 7.1µgS/g soil causing a lowering of the specific activity of the extractable sulphate, discussed above. This demonstrated the high rate of sulphur turnover also found in the Whitsome soil.

Although equilibrium, in the Stirling soil, between sulphur-32 and sulphur-35 in the various sulphur fractions was not reached after 75 days of incubation the specific activities of the portions of the various sulphur fractions undergoing transformation should be very similar. Such an assumption allows the estimation of the percentages of the soil sulphur fractions actively undergoing transformation in the Stirling soil (this assumption also holds for the Whitsome soil, assuming a similar rate of change). The results for both soils (Table 58) show that only a small fraction of the organic soil sulphur was actively undergoing transformation. This fraction will represent the labile soil sulphur pool, capable of rapidly replenishing levels of plant available sulphur. The HI-reducible fraction was more active than the carbon-bonded fraction in both soils. However this difference was much more marked in the Whitsome soil regardless of glucose-carbon addition. This is good evidence for supposing that HI-reducible sulphur is of a transitory nature and is an immediate precursor to sulphate-sulphur. Other workers have reached similar

conclusions about the HI-reducible fraction (Freney et al., 1971 and 1975; McLaren and Swift, 1977). The carbon bonded sulphur is less active than the HI-reducible sulphur in the Stirling soil and markedly less active in the Whitsome soil during the 75 day incubation but its role in the long term supply of sulphur to plants could be more significant.

4.5.3. Re-incubation of labelled Whitsome and Stirling series soils.

Portions of the two Whitsome soils (incubated with and without added glucose-carbon) and the Stirling soil labelled with sulphur-35 were leached with calcium chloride and re-incubated with added nutrients and a starter innoculum as described in section 3.7.1.1. The Whitsome soils were leached in leaching tubes then allowed to dry out over a few days to allow easy removal of the soil from the tube. The soil was then air-dried. However the Stirling soil was leached using a Buchner filtration unit and then immediately air-dried. This change in method was introduced because it was considered likely that the soil could mineralise sulphate while it was slowly drying in the leaching tubes. Extractable soil sulphate, sulphate-35 activity and the specific activity of the extractable sulphate of the leached soils were determined at 0, 7, 14, 21, 28 and 50 days incubation (Figs. 16, 17 and 18 respectively).

It can be seen that considerable quantities of extractable sulphate-32 and sulphate-35 are present even

Figure 16. Changes in extractable soil sulphur-32 levels with time in the Whitsome and Stirling soil series.

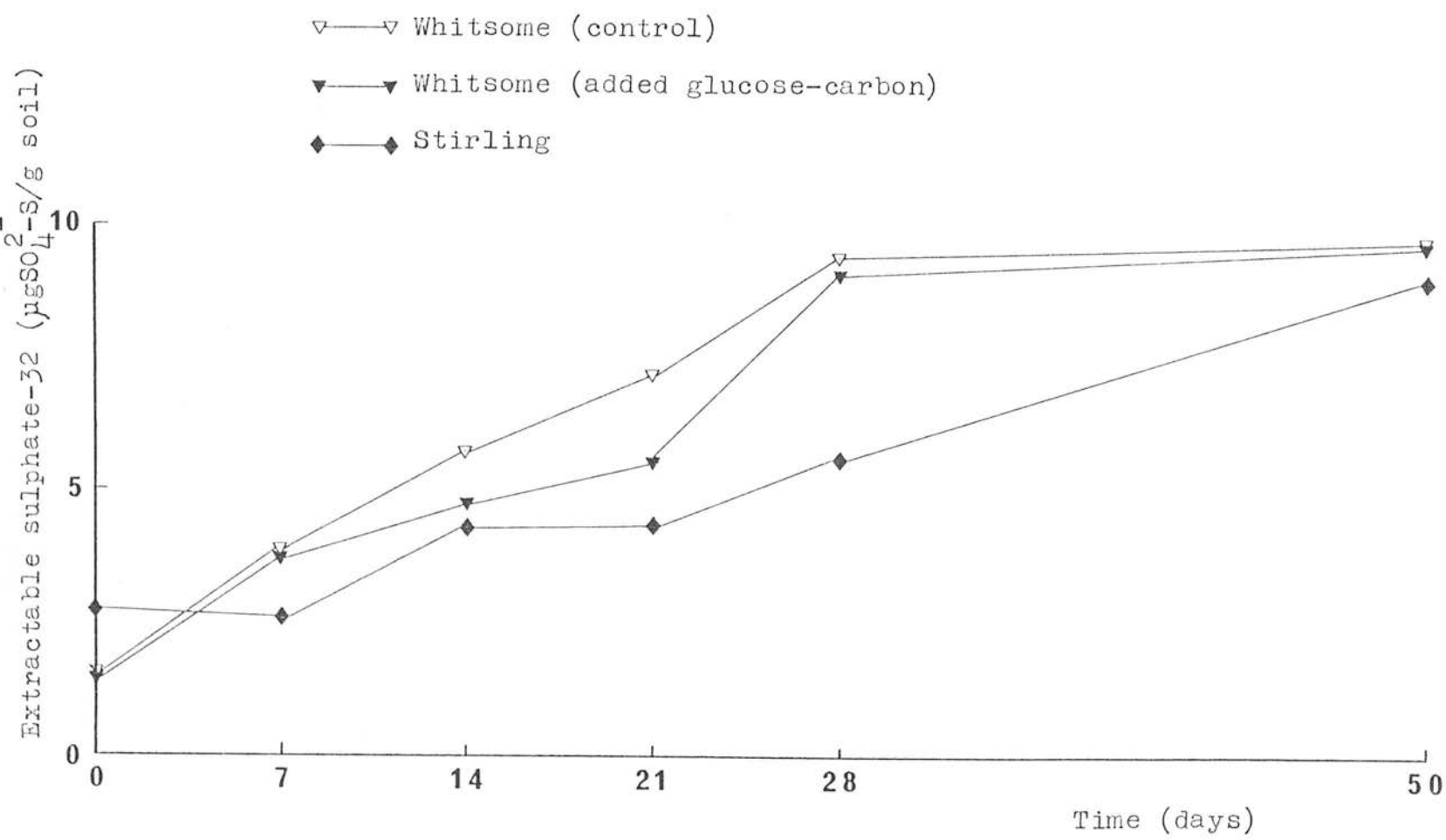


Figure 17. Changes in extractable sulphur-35 levels with time in the Whitsome and Stirling soil series.

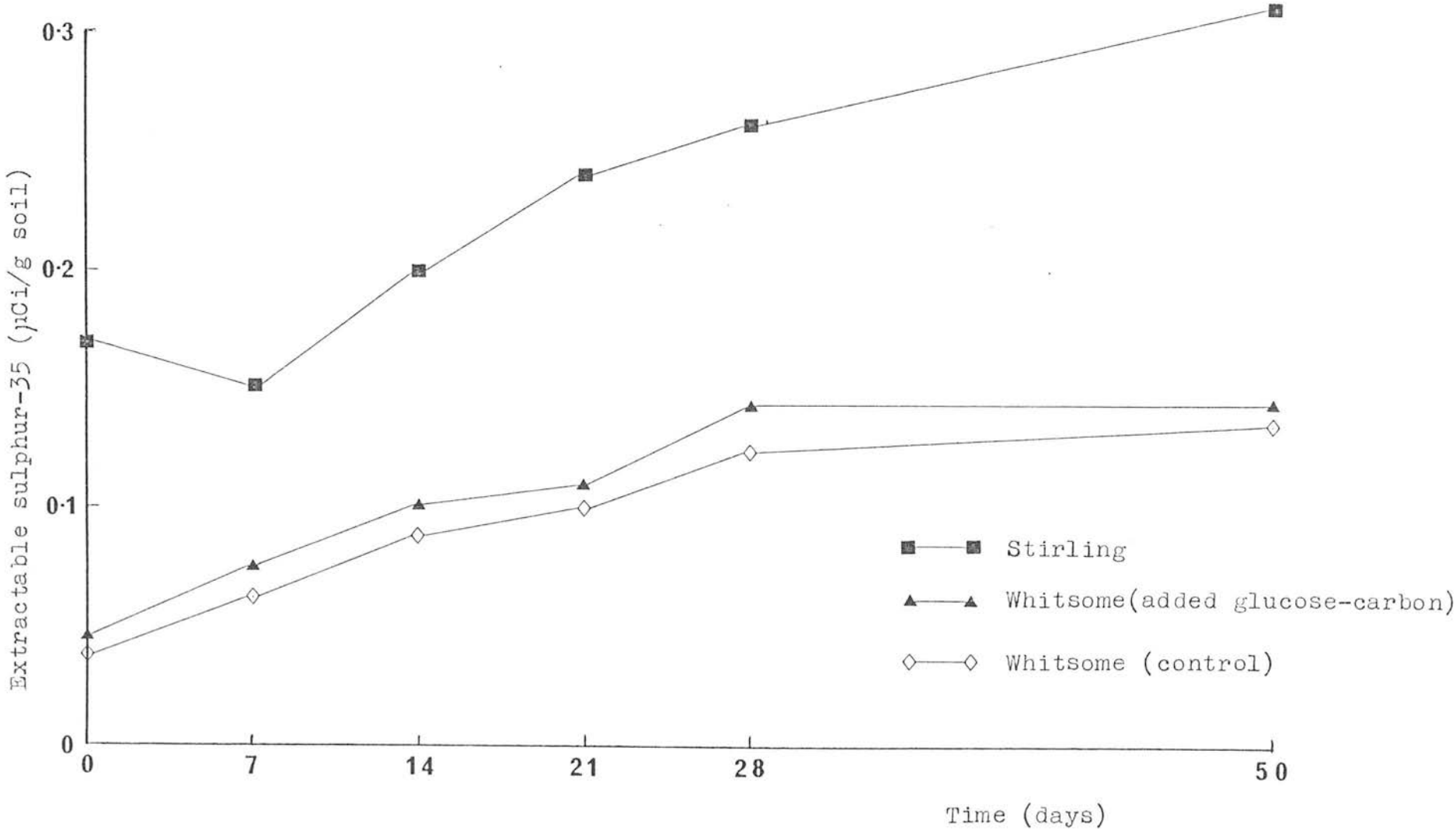
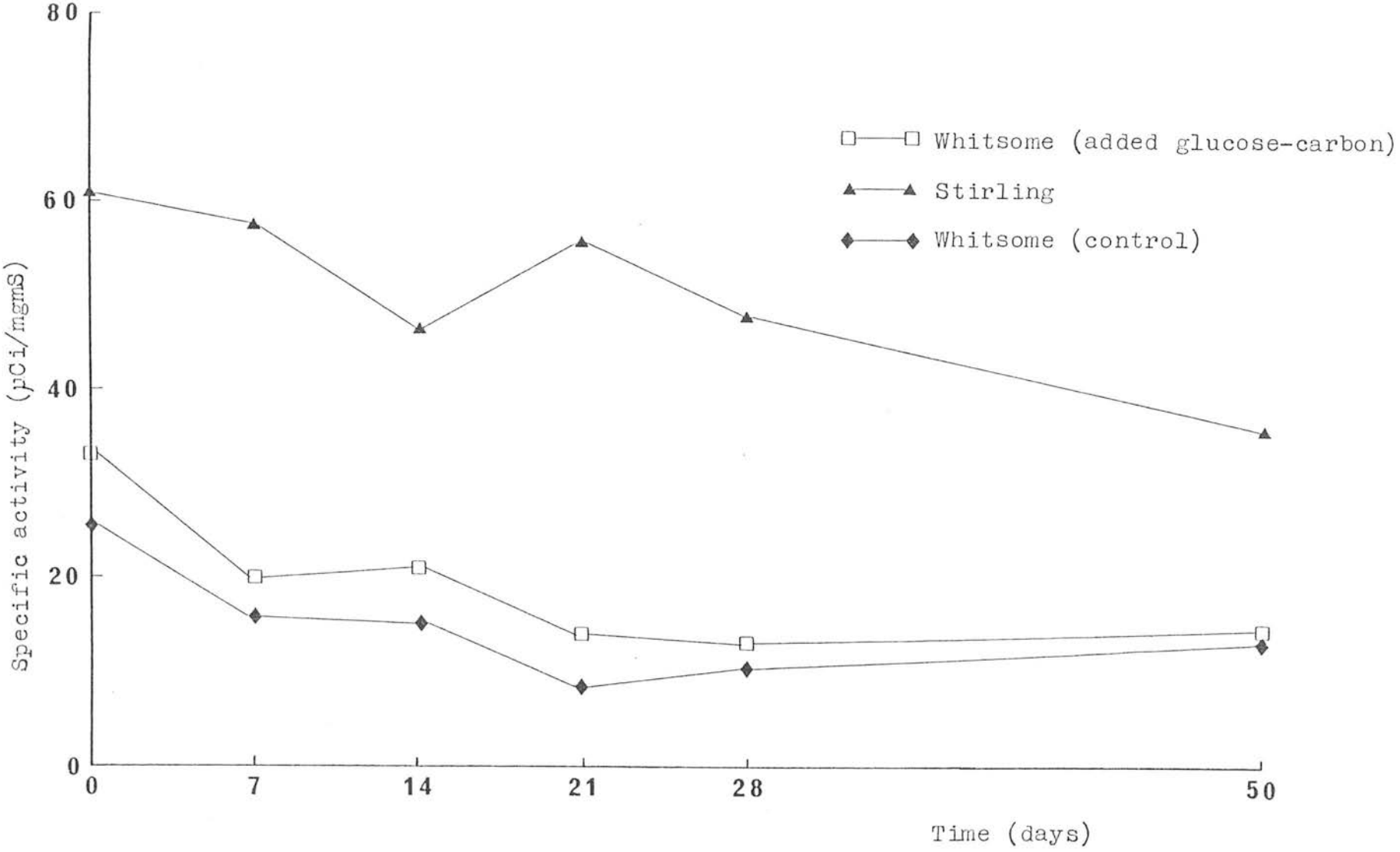


Figure 18. Changes in specific activity with time in the Whitsome and Stirling soil series



after thorough leaching of the soils (see sulphate levels at 0 days incubation). Since both the Whitsome and the Stirling soils show this characteristic it is probable that the air drying was instrumental in liberating quantities of sulphate-32 and sulphate-35 rather than the slow drying period employed with the Whitsome soil only. This effect of air-drying has been widely reported in the literature (Barrow 1960; Williams 1967). The sulphate released by air drying has a high specific activity compared to that of the total organic soil sulphur. This indicated that the more recently incorporated sulphur was more susceptable to breakdown into sulphate. Therefore the use of air-dried soils in sulphur-response type pot experiments could provide useful information about the soils reserve sulphur status. Criticism in the literature (Barrow, 1960) of the use of air dried soils for pot experiments is therefore probably unjustified.

All the soils showed a similar rate of sulphur-32 mineralisation which was very different to that exhibited by a range of soils in section 4.4. The initial flush of mineralised sulphate, previously observed, was absent due probably to the effect of leaching which will remove readily utilisable substrates and nutrients and affect the numbers and balance of microbial populations. All soils show a steady increase, with time, in levels of mineralised sulphate-32 although only the Whitsome soils appear to attain an equilibrium within the 50 days incubation.

TABLE 59. The amounts of sulphur-32 and sulphur-35 mineralised from re-incubated Whitsome series soil.

a) Soil originally incubated with added glucose-carbon

Incubation period (days)	Mineralised S-32 ($\mu gSO_4^{2-}S/g$)	Mineralised S-35 ($\mu Ci/g$)	Specific Activity of mineralised S ($\mu Ci/mgmS$)
0	1.4	0.046	32.9
7	3.7	0.074	20.0
14	4.7	0.099	21.1
21	5.5	0.107	19.4
28	9.0	0.141	15.7
50	9.5	0.139	14.6

b) Soil originally incubated without added glucose-carbon

Incubation period (days)	Mineralised S-32 ($\mu gSO_4^{2-}S/g$)	Mineralised S-35 ($\mu Ci/g$)	Specific Activity of mineralised S ($\mu Ci/mgmS$)
0	1.5	0.038	25.3
7	3.8	0.062	16.2
14	5.7	0.087	15.3
21	7.1	0.100	14.1
28	9.3	0.122	13.1
50	9.5	0.134	14.1

All values above represent the mean of two determinations made on duplicate treatments.

TABLE 60. The amounts of sulphur-32[¥] and sulphur-35 mineralised from re-incubated Stirling series soil

Incubation period (days)	Extractable sulphate-32 (μgS/g)	Extractable sulphur-35 (μCi/g)	Specific Activity of extractable sulphur (μCi/mgmS)
0	2.8	0.17	60.7
7	2.6	0.15	57.7
14	4.3	0.20	46.5
21	4.3	0.24	55.8
28	5.5	0.26	47.3
50	8.8	0.31	35.2

[¥]reported values represent the mean of two determinations made on two replicate treatments.

For the Stirling soil, the first 7 days of incubation showed a net immobilisation of both sulphate-32 and sulphur-35. It is difficult to explain exactly why immobilisation occurred since leaching and air-drying will greatly disrupt the balance of easily utilisable carbon, nitrogen and sulphur. The effect of the glucose-carbon added to the Whitsome soil can be seen in Figs. 16, 17 and 18. The soil receiving glucose carbon mineralised sulphate-32 at a similar rate to the control soil but the amounts of sulphur-32 mineralised at each sampling date were always less. However the soil which received glucose-carbon mineralised greater amounts of sulphur-35 than the control soil. This is because the glucose-carbon treated soil possessed a greater proportion of labelled organic sulphur. For all the soils the changes in sulphate-32 levels closely paralleled changes in sulphur-35 levels.

Re-incubation immediately reduced the specific activity of the extractable sulphate of all the soils used. This is due to mineralisation of unlabelled organic sulphur compounds causing isotopic dilution in the extractable sulphur pool. In the Stirling soil the specific activity of the extractable sulphate decreases throughout the 50 days of incubation whereas the Whitsome soils appear to attain a constant specific activity after 28 days incubation. It is most interesting to note that the specific activity of the extractable sulphate of both soils at any stage of the incubation was markedly higher

than the specific activity of the total organic soil sulphur. For example after 50 days incubation of the Stirling soil the specific activity of the extractable sulphate was 35.2μCi/mg S, compared to the specific activity of the total soil sulphur before leaching which was 2.25μCi/mg S. This demonstrated that the more recently incorporated soil sulphur is the most easily mineralisable fraction. This finding provides a means of re-evaluating traditional soil sulphur extracting solutions by firstly extracting a labelled soil and then comparing the specific activities of the extracted sulphur and the total soil sulphur. A means is thus available, to develop an extractant capable of predicting the soils reserve sulphur status.

The final specific activities of the extractable sulphate in all the soils was considerably lower than the specific activity of the soil extractable sulphate prior to leaching and air drying. This could be due to the air drying which liberates labile sulphur-32, the subsequent mineralisation of which, dilutes the sulphur-35 present in the extractable soil sulphate. This modification of the soil sulphur could explain the continual decrease in extractable sulphate specific activity and non-attainment of equilibrium in the Stirling soil.

4.6 Fractionation of soil organic sulphur

Since soil organic matter consists of an infinite number of organic compounds of varying molecular weight

and chemical compositions it is almost certain that, similarly, a vast range of different sulphur containing organic molecules are present in the soil. The complexity and vast range complicates the isolation of individual compounds. Therefore the approach adopted here was to fractionate the extracted sulphur compounds on a basis of molecular weight and characterise these fractions in terms of chemical composition and susceptibility to mineralisation. Greater knowledge of the soil organic sulphur will aid the development of methods capable of assessing the long term sulphur supplying power of soils.

This section begins by examining a previously little used soil sulphur extraction procedure. Methods used to fractionate the extracted sulphur are then outlined together with attempts to characterise the fractions.

4.6.1 Extraction of soil organic sulphur

Many methods are available for the extraction of soil organic sulphur. However most methods either extract very small percentages of the total soil sulphur or the reagents employed are liable to alter the forms of sulphur extracted. An extraction procedure which successfully overcomes these problems was reported by Scott and Anderson (1976a). This method, described below, was adopted for the work here with only slight modification. Finely ground soil (2g or 10g of $<$ 100 mesh) was shaken twice with two 40 ml portions of 0.1M hydrochloric acid and then with water for five minutes. The acid pre-

treated soil was then added to 90 ml of 0.2M acetylacetone (previously adjusted to pH8 with sodium hydroxide) and left standing overnight. After standing, the mixture was re-adjusted to pH8 with 3M sodium hydroxide. The soils were then dispersed for 10 minutes using a 150 Watt ultrasonic disintegrator (MSE Scientific Instruments) fitted with a 19mm end diameter, titanium probe. The ultrasonified extracts were centrifuged at 8,500g for thirty minutes, the supernatant decanted, and the residue was treated again with 0.2M acetylacetone at pH8. The two extracts were pooled and made up to 200 ml with distilled water. Extracts were stored at $4^{o}C$.

Initially the extraction method was investigated using Kilmarnock arable, Kilmarnock pasture, Stirling arable and Stirling pasture soils. Two soil/extractant ratios (1:45 and 1:9) were examined (Table 61(a) and (b)). Acetylacetone, used in conjunction with ultrasonic disintegration at a soil:extractant ratio of 1:45, extracted 78-100% of the total soil sulphur from the four soils investigated. Lowering the soil:extractant ratio to 1:9 reduced the percentage of the soil sulphur extracted to 58-89%. Although soil sulphur is more efficiently extracted using the 1:45 soil:extractant ratio, for purely practical reasons large quantities of extracted organic sulphur were obtained more conveniently using the 1:9 ratio.

Large quantities of extracted soil sulphur were required for the chemical analysis of fractions. Where

TABLE 61. Amounts of soil sulphur extracted by 0.2M acetylacetone at pH8

(a) Soil:extractant ratio of 1:45 and ultrasonic dispersion

Soil	Total sulphur (µgS/g)	Extracted sulphur (µgS/g)	% of total sulphur extracted
Kilmarnock arable	352	280	80
Kilmarnock pasture	705	550	78
Stirling arable	530	530	100
Stirling pasture	632	640	101

(b) Soil:extractant ratio of 1:9 and ultrasonic dispersion

Soil	Total sulphur (µgS/g)	Extracted sulphur (µgS/g)	% of total sulphur extracted
Kilmarnock arable	352	250	71
Kilmarnock pasture	705	392	56
Stirling arable	530	474	89
Stirling pasture	632	424	67

(c) Soil:extractant ratio of 1:45. No ultrasonic dispersion

Soil	Total sulphur (µgS/g)	Extracted sulphur (µgS/g)	% of total sulphur extracted
Kilmarnock arable	352	130	37
Kilmarnock pasture	705	420	60
Stirling arable	530	260	49
Stirling pasture	632	260	41

TABLE 61 (contd.)

(d) Soil:extractant ratio of 1:9. No ultrasonic dispersion

Soil	Total sulphur (µgS/g)	Extracted sulphur (µgS/g)	% of total sulphur extracted
Kilmarnock arable	352	128	36
Kilmarnock pasture	705	166	24
Stirling arable	530	288	54
Stirling pasture	632	238	38

All values above are the mean of duplicate determinations.

TABLE 62. A comparison between the forms of extracted soil sulphur and the forms of indigenous soil sulphur

Soil	Soil: extractant ratio	Soil HI reducible S as % of total soil S	Extracted HI reducible S as % of total S extracted
Kilmarnock arable	1:45	53	48
Kilmarnock pasture	1:45	41	40
Stirling arable	1:45	52	46
Stirling pasture	1:45	50	47
Kilmarnock arable	1:9	53	60
Kilmarnock pasture	1:9	41	50
Stirling arable	1:9	52	58
Stirling pasture	1:9	50	54

more soil is present (in the 1:9 soil:extractant ratio) a reduction in the efficiency of the ultrasonic disintegrater probably causes the depression in yields of extracted sulphur. The amounts of sulphur extracted from the Kilmarnock and Stirling soils were very similar to those reported by Scott and Anderson (1976) for other soils. The effect of the ultrasonic disperson treatment was examined by comparing percentage extraction data with and without ultrasonic dispersion (Table 61(c) and (d). Results show that the ultrasonic dispersion greatly increased the amounts of sulphur extracted at both soil/extractant ratios. Only 37-60% of the soil sulphur was extracted at the soil/extractant ratio of 1:45 and 24-54% at the 1:9 soil/extractant ratio.

Scott and Anderson (1976a) suggested that relatively unaltered forms of soil organic sulphur were extracted by the acetylacetone procedure. Further evidence to support this claim is presented in Table 62. The ratio of HI-reducible sulphur to carbon-bonded sulphur in the extract remains very similar to that in the whole soil. Since the two sulphur fractions are extracted in a similar ratio to which they occur in the soil two assumptions can be made:-

(i) both fractions are equally easily extracted.

(ii) extraction processes do not cause conversion between the HI-reducible and carbon-bonded sulphur forms.

TABLE 63. A comparison between the C:S ratio of the acetylacetone extract and the soil before extraction*

Soil	C:S ratio of soil	C:S ratio of extract
Kilmarnock arable	68	45
Kilmarnock pasture	66	40
Stirling arable	40	16
Stirling pasture	88	49

*a soil:extractant ratio of 1:45 and ultrasonic dispersion were used.

The carbon:sulphur ratios of an extract, obtained using ultrasonic dispersion and a soil:extractant ratio of 1:45 were determined (Table 63). The C:S ratio of the extract was markedly narrower than that of the whole soil. This indicates that acetylacetone extracted relatively more sulphur than organic carbon.

4.6.2 The development of gel chromatography for use with soil organic sulphur extracted by acetylacetone

Gel permeation chromatography is a biochemical technique widely employed to purify and separate macromolecules. Under certain operational conditions molecules will be eluted from a column of gel according to their molecular weight. This separation depends upon the ability of the solute to enter the pores of the stationary phase. The larger molecules which will not enter into the gel pores (excluded portion) will be eluted first while the smaller molecules will be held up by the gel phase (included portion). Since molecules are eluted in order of decreasing molecular size careful selection of gel type enables separation of molecules of a given molecular weight. However since the separation is strictly based on molecular size the molecular weight exclusion limit of a gel is nominal and will depend largely upon molecular structure.

Soil sulphur, extracted using acetylacetone, has been fractionated previously by gel chromatography (Scott and Anderson, 1976). However these workers employed water as the eluant which, according to Swift and Posner (1971),

TABLE 64. Operating conditions used to fractionate soil organic sulphur by gel filtration

	Gel Type	Eluant	Flow Rate (cm^3/hr)	Column Length (cm)	Void Volume (cm^3)	Total Column Volume (cm^3)	Sample - preparation and application
System I	G100	Distilled water	10	49	105	255	10g soil was extracted with acetylacetone as described above. The extract was made up to 200ml - 100ml of this extract was rotary-film evaporated down to 25ml - this was applied to the column after passing through a 200µm filter.
System II	G100	Sodium carbonate/ bicarbonate buffer at pH 8.9	20	47	90	250	5ml of a 10g soil in 200ml soil extract was applied to the column surface after passing through a 2.2µm filter.
System III	G100	Sodium carbonate/ bicarbonate buffer at pH 8.9 in sodium chloride(0.05M)	20	47	90	250	20ml of a 10g soil in 200ml soil extract was extracted in ether, passed through a 2.2µm filter, reduced in volume to 10ml and applied to the column surface.
System IV	G200	Tris* buffer at pH 9	40	38.5	290	756	100ml of a 10g soil in 200ml soil extract was extracted in ether, reduced to 30ml, passed through a 2.2µm filter and applied to the column surface.

*Tris = 2-amino-2 (hydroxymethyl-propane-1,3 diol.)

leads to gel-solute interactions. Where such interactions occur a separation on the basis of molecular weight alone is not achieved. It was therefore decided to compare the performance of a series of gel/eluant combinations with acetylacetone extracts. Experimental details of the systems which incorporate the use of Sephadex G type gels (Pharmacia Uppsala, Sweden) are given in Table 64. The systems are listed in chronological order of examination and the table represents the development of a suitable fractionation system. Void volumes were determined using Blue Dextran 2000 (Pharmacia, Sweden). Some details of sample preparation are given in Table 64 but the 1:9 soil: extractant ratio was used throughout with the pooled extracts made up to 200 ml in a volumetric flask. The extracts were filtered using a 2.2µm filter (Millipore, France). Whenever rotary film evaporation was used to reduce the volume of soil extracts the temperature was kept below 40°C to minimise transformation of the sulphur forms. Where appropriate, ultrafiltration equipment (Amicon, U.S.A.) was used in preference to rotary evaporation in order to lessen the possibility of pyrolysis of organic sulphur compounds. Eluant fractions of 5g were collected throughout, using a rectangular balance-operated fraction collector (Toyo, Japan). The elution of organic matter from the gel column was monitored by determining the optical density of each fraction at 410 nm.

The first set of operating conditions, using water as the eluant, (System I) gave problems of organic matter adsorption (reversible and irreversible) onto the gel. Staining of the gel and elution of organic material outside the total column volume were indicative of adsorption. Therefore separation was not related to molecular weight. Also the column can only be used once since adsorbed material continued to be slowly eluted. Subsequent elutions of the same soil extract gave improved recoveries of organic matter, indicating that perhaps sites of irreversible adsorption had been filled during the elution of former samples. These factors gave rise to work which would have been difficult to repeat and would not have allowed comparison of different soil extracts eluted down the same gel column.

The excluded fractions were opalescent in appearance indicating a markedly colloidal character whilst the included fraction was a clear reddish-orange colour. The colloidal properties of the high molecular-weight material was probably caused by contamination with fine clay. To enable more comparative work to be undertaken a second system was proposed, using an alkaline buffer as eluant (System II, Table 64).

The use of a buffer at pH8.9 decreased irreversible adsorption (less staining of the gel occurred) but some reversible adsorption occurred since organic material was eluted outside the total column volume. The buffer gave a different elution pattern to the water which

would be more related to molecular weight. The bright red colouration of the included fraction indicated that iron-acetyl acetone complexes were present. This was confirmed by comparing the U.V. absorption spectra of a laboratory prepared solution of iron-acetylacetone complex with that of the included fractions. Both the included material and the laboratory prepared iron-acetylacetone complex gave a peak at 280 nm whilst the excluded material showed no peak at this wavelength. Therefore if the distribution of molecular weights of the organic compounds is to be assessed by monitoring the optical density of the eluant, the coloured iron complexes must be removed. It has been shown that diethyl ether extraction of the organic matter extract will remove the iron complexes (Halstead et al., 1966). Attempts to use diethyl ether here for this purpose also proved effective. Analysis of the non- aqueous phase showed that no sulphur had been removed from the organic matter extract. The extract appeared to be more colloidal after ether extraction and possessed a much darker brown hue.

The third gel system examined (System III, Table 64) employed a larger sample of soil extract which had been previously extracted with diethyl ether. This system of gel filtration allows comparison between the elution patterns of different soil organic matter extracts. The only problem with this system is that optical density gives a poor indication of the amount of organic matter

occurring in the excluded fraction since fine clay will cause dispersion of the light thereby greatly increasing the observed optical density. Figures 19 and 20 show that cultivation has not greatly affected the molecular weight distribution in the organic matter extract. However cultivation has lowered the organic matter level in the soil and hence the amounts of organic matter extracted by acetylacetone. To test whether this system truly fractionated on a basis of molecular weight the elution patterns, obtained by applying to the column two different amounts of Kilmarnock (arable) soil organic matter extract, were compared. Swift and Posner (1971) claimed that the elution pattern should be independent of sample size and sample concentration. It was found that the elution patterns, for the 20 ml and 40 ml soil extract applications were almost identical. (55 percent of the 40 ml application was excluded and 54 percent of the 20 ml application was excluded). Thus the system allowed the determination of reproducible molecular-weight distributions for organic matter extracts. However the amounts collected in any one fraction were very small and did not allow subsequent chemical analysis.

A fourth system was examined (System IV, Table 64) to enable larger amounts of organic matter to be fractionated at one time. Sephadex G200 was used in this case to increase the amount of organic matter occurring in the included portion. Tris buffer at pH9 was used because alkaline buffers containing an amino cation have been recommended for use with gel chromatography of soil

Figure 19. The fractionation of soil organic matter extracts, obtained from Kilmarnock soil series, using Sephadex G100.

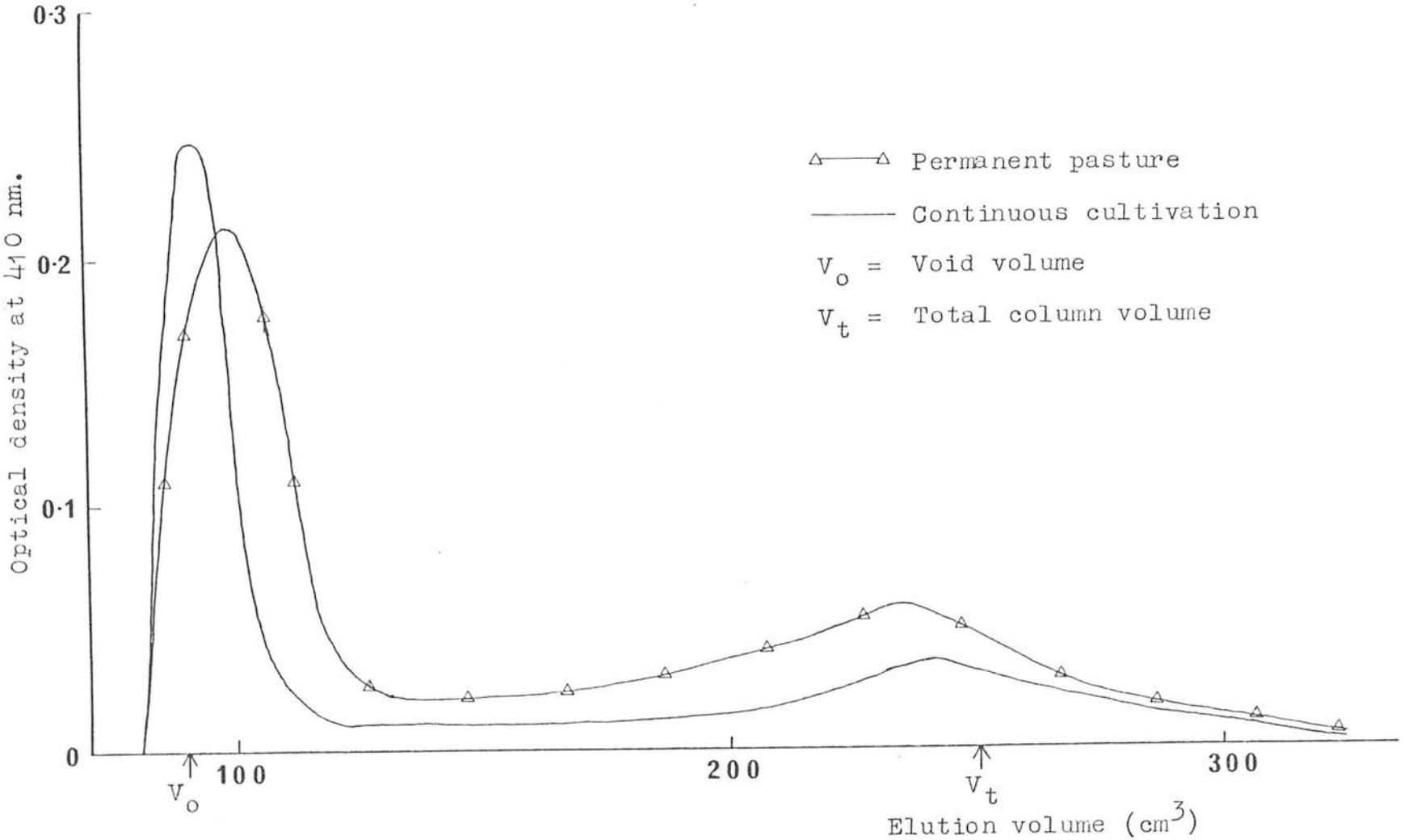
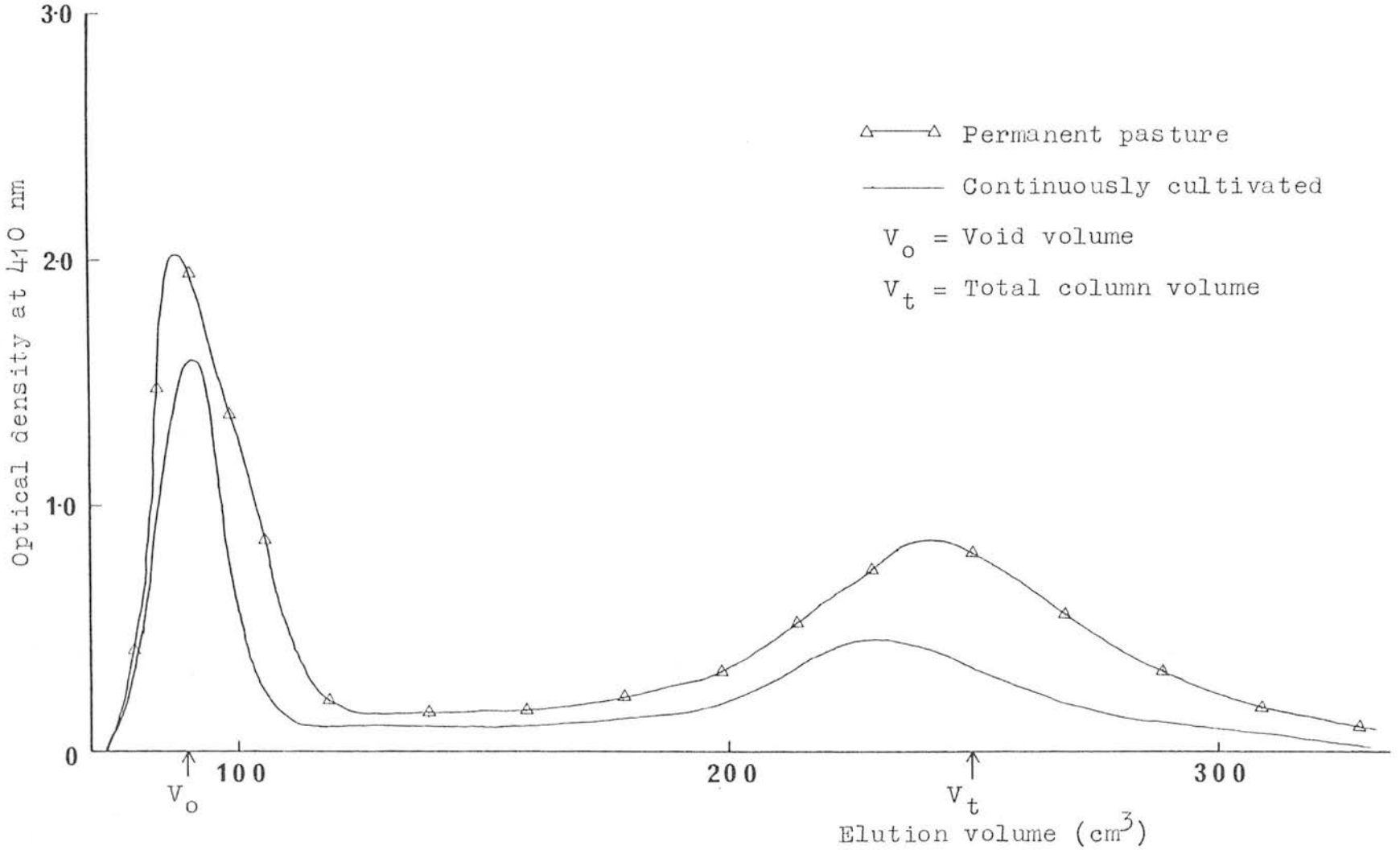


Figure 20. The fractionation of soil organic matter extracts, obtained from Stirling soil series, using Sephadex G100.



organic matter (Swift and Posner, 1971). Work reported here also showed that this buffer reduced gel/solute interactions and worked well in conjunction with Sephadex G200 and large applications of soil extract. This system formed the basis of a fractionation scheme described in the following section.

4.6.3 Fractionation of soil organic sulphur on the basis of molecular weight and chemical form.

Arable and cultivated soils of the Kilmarnock and Stirling soil series were extracted with acetylacetone as described in section 4.6.1. Portions of organic matter extracted from each soil were eluted down a column of Sephadex G200 according to the conditions of System IV (Table 64). The excluded material (molecular weights greater than 200,000) was dialysed using Visking tubing (Scientific Instrument Centre Ltd., London) to remove the salt contained in the buffer. The organic material was then freeze-dried, weighed and retained for analysis.

The included organic material (molecular weights less than 200,000) was re-fractionated by the conventional humic/fulvic type of separation. This entailed adjustment of the included organic material to pH1 using 2.5M hydrochloric acid. The precipitated humic acid was centrifuged down at 1500 r.p.m., washed with 50 ml of distilled water and re-centrifuged. The humic acid was slurried with 100 ml of distilled water and freeze-dried. The fulvic acid (the soluble fraction at pH = 1) was re-adjusted to pH 7 with M Na OH, dialysed to remove salts

and then freeze-dried. Yields of humic and fulvic acids were recorded and the freeze-dried material retained for analysis.

The various organic matter fractions were analysed for ash content, total sulphur, HI-reducible sulphur and carbon-bonded sulphur (by difference). Results are shown in Figures 21 - 24.

It can be seen (Fig. 21) that the fractions containing organic material above 200,000 molecular weight also contained much mineral matter. This is due to the high content of fine clay which passed the 2.2 µm millipore filter. This fine clay is probably intimately associated with the organic matter which occurred in this fraction (mechanisms have been suggested by Greenland (1971)). The humic acid < 200,000 molecular weight is almost ash free but the fulvic acids contain as much as 40% ash, probably due to the presence of sodium and aluminium intimately associated with the organic matter. The long term cultivation imposed on two of the soils enhanced mineralisation of the organic matter leading to lowered organic matter levels (and hence lower sulphur levels since the organic matter is essentially the sole source of sulphur in these soils). This phenomenon is reflected in the amounts of organic matter extracted from the Kilmarnock pasture soil, which is greater than the amount of organic matter extracted from the Kilmarnock arable soil. Cultivation has reduced all three organic matter fractions in the Kilmarnock soil but more

especially the humic acid < 200,000 and the organic matter > 200,000 molecular weight. The effect of cultivation is not seen in the Stirling soils where similar amounts of organic matter were extracted from the pasture and cultivated soils.

Figures 22 and 23 respectively show the concentrations and amounts of sulphur found in the organic matter fractions of each of the four soils. The highest sulphur concentrations for all soils are found in the fulvic acid < 200,000 molecular-weight fraction. Cultivation of the Stirling soil has reduced the sulphur level in the more labile fulvic acid < 200,000 molecular-weight fraction whereas in the Kilmarnock soil there is no reduction. The levels of sulphur in the > 200,000 molecular-weight fraction remain unaltered by cultivation in both soils. For both soils, cultivation has increased the concentrations of sulphur in the humic acid < 200,000 molecular-weight fraction suggesting that non-sulphur components of the fraction are more easily mineralised. McLaren and Swift (1977) and Bettany et al., (1980) have found that sulphur is more resistant to mineralisation than both carbon and nitrogen.

For the Kilmarnock soils, the greatest amount of sulphur was found in the > 200,000 molecular-weight fraction. Although only small amounts of sulphur were recovered from the humic acid < 200,000 molecular-weight fraction, much of the sulphur found in the > 200,000 molecular-weight fraction would also have occurred in

humic acid associated with this fraction. Cultivation of the Kilmarnock soil has resulted in the loss of sulphur from all three fractions but more especially from the > 200,000 molecular-weight and humic acid < 200,000 molecular weight fractions. This, however is an over simplification since cycling of sulphur forms between the three fractions was likely to have occurred during mineralisation. The Stirling soil differed from the Kilmarnock soil in that the largest amounts of sulphur occur in the < 200,000 molecular weight fractions. The effect of cultivation on the Stirling soil was only seen in the fulvic acid fraction < 200,000 molecular-weight where cultivation has caused the mineralisation of much fulvic acid sulphur. The amounts of sulphur in the other two fractions remained the same after cultivation.

The chemical forms of sulphur occurring in the organic matter fractions were investigated by determining the relative proportion of HI-reducible sulphur and carbon-bonded sulphur in each fraction (Fig. 24). It was very noticeable in all soils that the majority of the > 200,000 molecular weight fraction occurred as HI-reducible sulphur. Bettany et al., (1979 and 1980) noted that the clay associated humic acid contained a higher percentage of HI-reducible sulphur than the humic acid solubilised by sodium hydroxide and sodium pyrophosphate. The clay associated humic acid of Bettany et al., (1979 and 1980) would be very similar to the > 200,000 molecular-weight material. Although it was

Figure 21. The yield and ash contents of the organic matter fractions obtained from Stirling and Kilmarnock soil series.

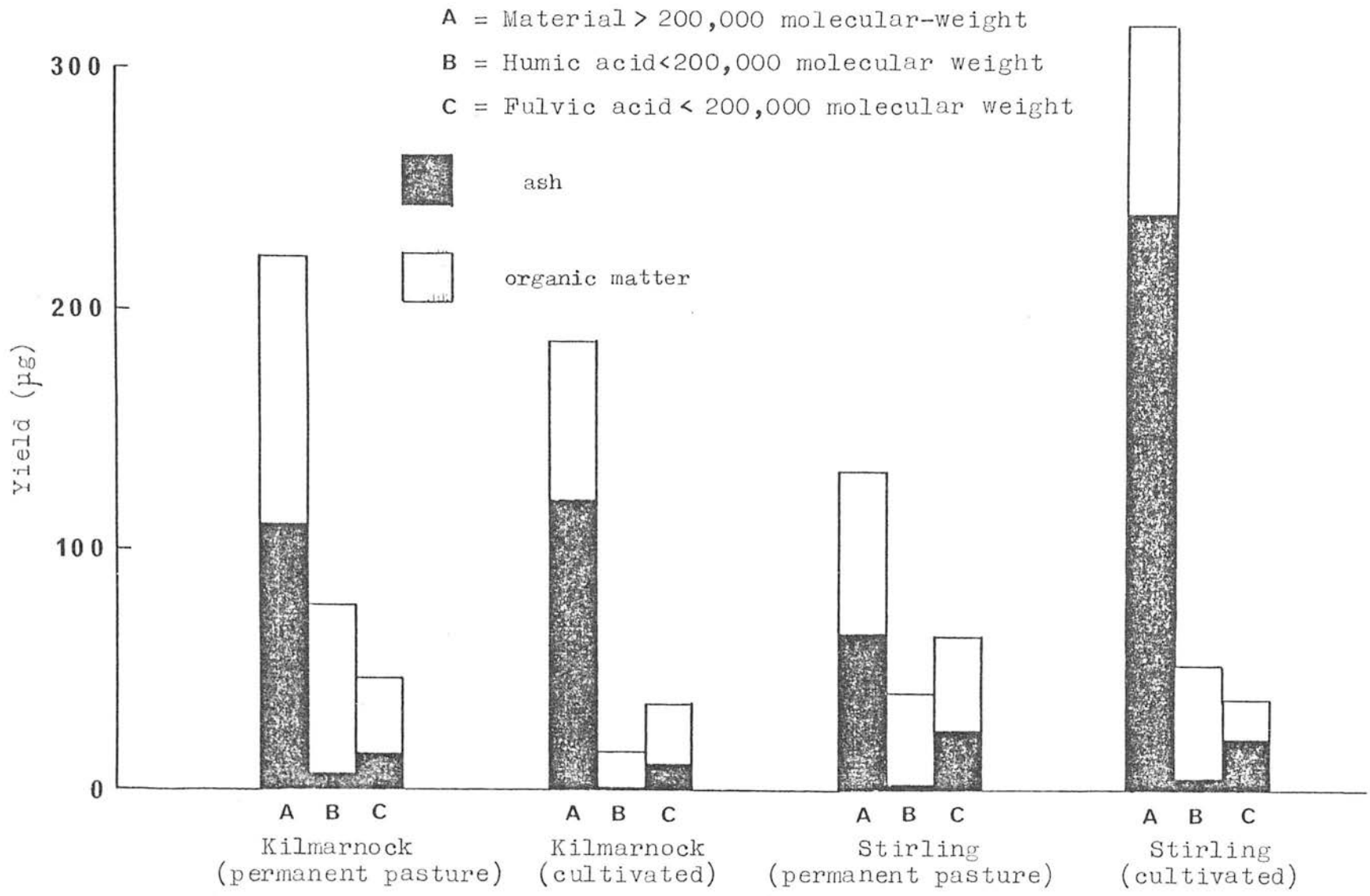


Figure 22. Sulphur concentrations in the organic matter fractions obtained from the Stirling and Kilmarnock soil series.

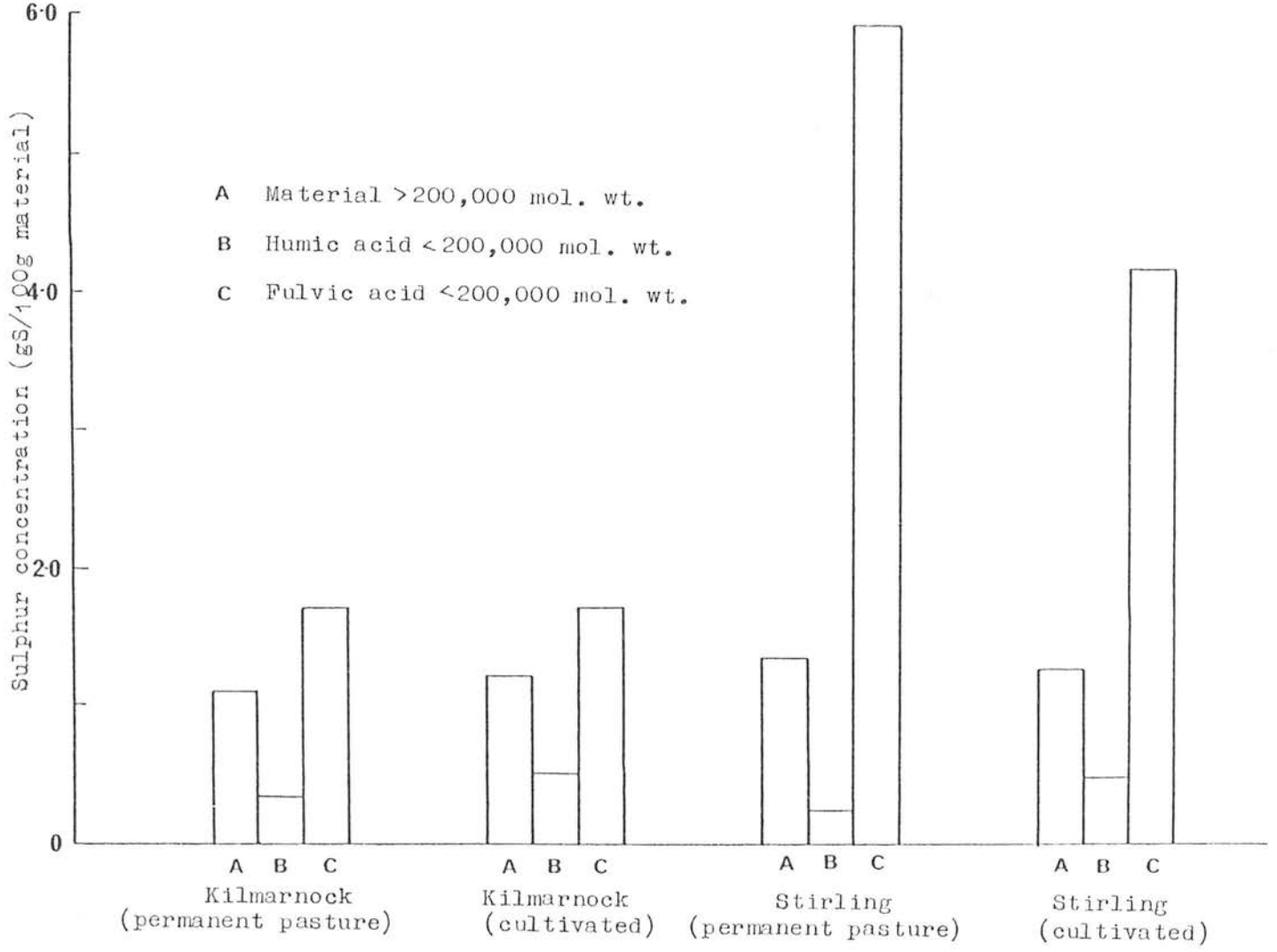


Figure 23. The amounts of sulphur recovered from organic matter fractions obtained from the Stirling and Kilmarnock soil series.

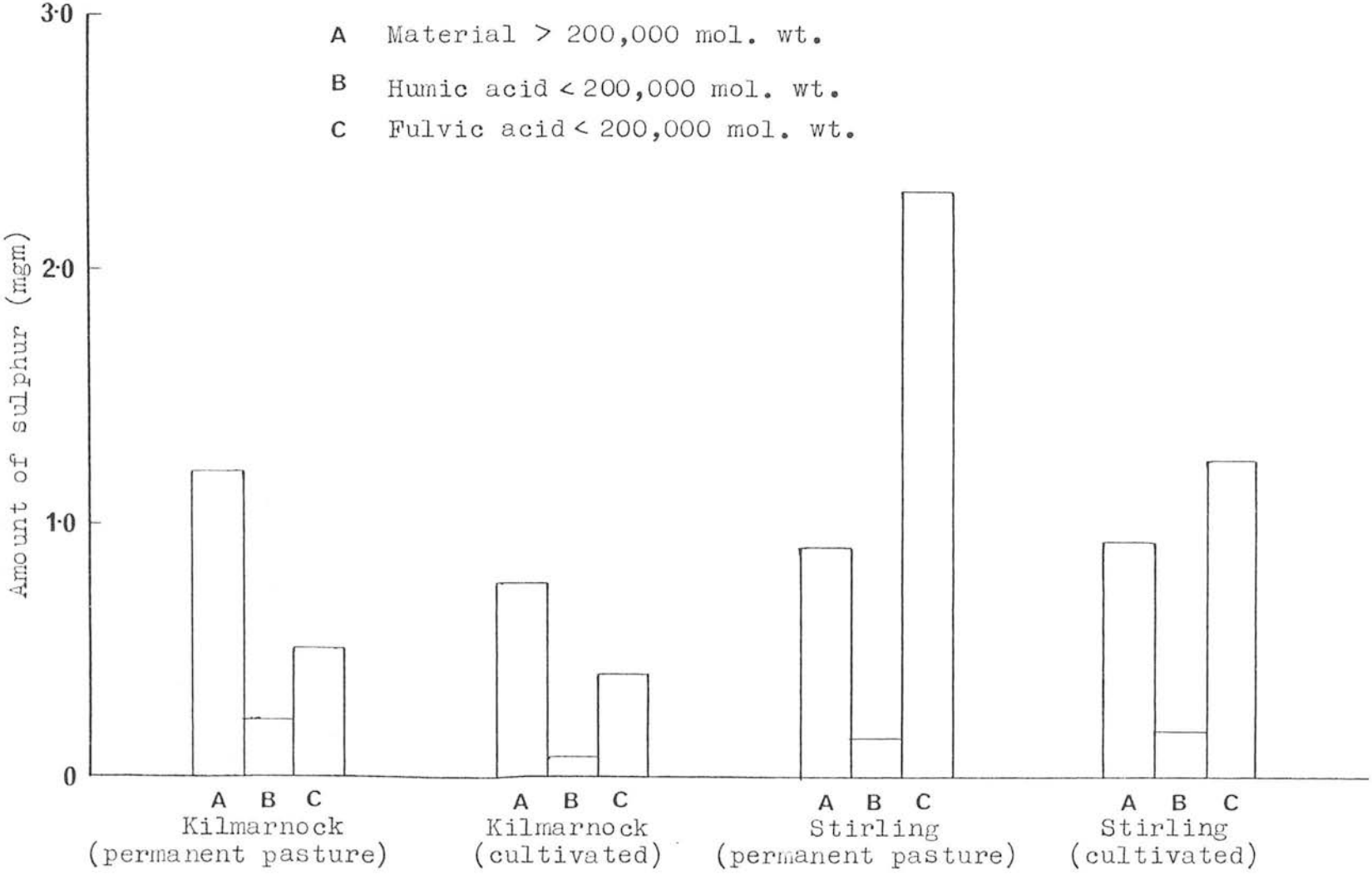
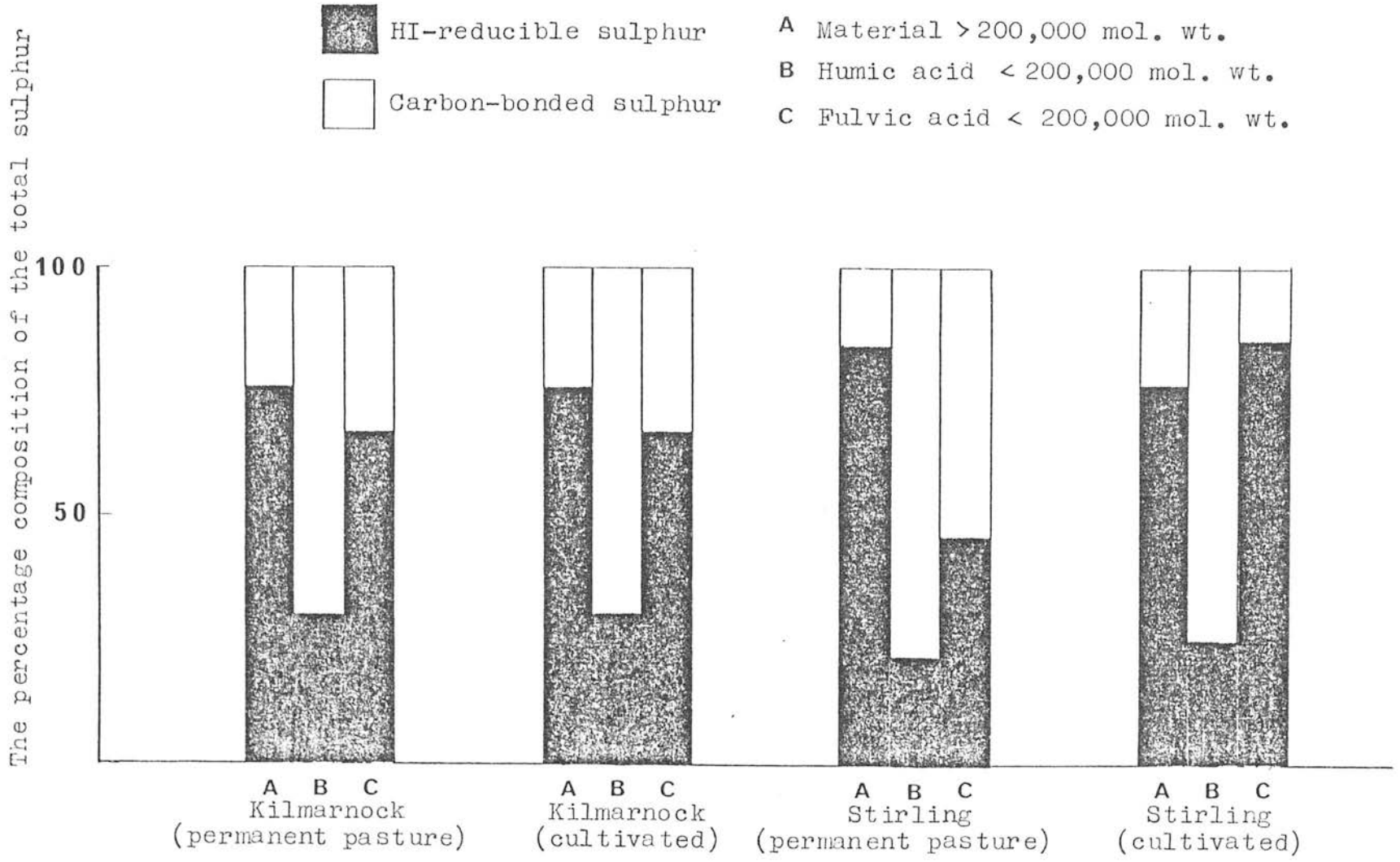


Figure 24. The chemical forms of sulphur in the organic matter fractions obtained from Stirling and Kilmarnock soil series.



initially surprising to find the transitory HI-reducible sulphur forms in the > 200,000 molecular weight fraction it is probable that the intimate association of this sulphur with the fine clay protects it from microbial transformation (Bettany et al., 1979). However the sulphur in the > 200,000 molecular-weight fraction could also occur as high molecular weight compounds which are HI-reducible:- for example sulphated polysaccharides. The fulvic acid < 200,000 molecular weight fractions in all the soils also contained a high proportion of HI-reducible sulphur. This agrees with reports that fulvic acid is more oxidised, is of lower molecular weight and is of a transitory nature. Since these are probably also properties of HI-reducible sulphur it is not surprising to find large percentages of HI-reducible sulphur showing up in the fulvic acid < 200,000 molecular-weight fraction. The humic acid < 200,000 molecular weight fraction gave the lowest percentages of HI-reducible sulphur for all four soils (26%-30% HI-reducible sulphur). Again this result agrees with work by Bettany et al., (1979). These workers used a different extractant but also found that humic acid sulphur was largely carbon-bonded and proposed that "HI-reducible sulphur is not readily incorporated into the condensed aromatic units" which make up the humic acid. It is worthwhile noting that the work reported here and that published by Bettany et al., (1979 and 1980) shows good agreement even though different soils and different

extracting procedures have been used. Both pieces of work showed that much HI-reducible sulphur occurred in the fraction intimately associated with colloidal clay and in the fulvic acid fraction.

4.6.4 The molecular-weight fractionation of organic sulphur extracted from Stirling series soil.

Stirling series soil, which had been under permanent pasture, was extracted with acetylacetone as described in section 4.5.1. The extract was extracted with diethyl-ether, passed through a 0.65 µm "Millipore" filter and applied to a series of Sephadex G and Sepharose gels. The extract was first eluted down a Sephadex G10 column but insufficient organic matter was included to allow subsequent freeze-drying and chemical analysis of this < 700 molecular-weight fraction. Similarly insufficient material was included after elution of the extract down a G-25 column and the small $< 5,000$ molecular-weight fraction was returned to the bulk of the extract. Therefore the first fraction collected was that included from the elution of the extract down a G-50 column. The excluded material from this elution was then fractionated as described in the flow chart (Fig. 25). The eluant used throughout was tris buffer at pH9. Table 65 shows some chemical properties of the Stirling soil organic matter fractions.

The molecular-weight distribution of the extracted organic matter can be examined by noting the amounts of ash free material in each molecular-weight fraction.

FIG. 25. The fractionation of soil sulphur according to molecular weight

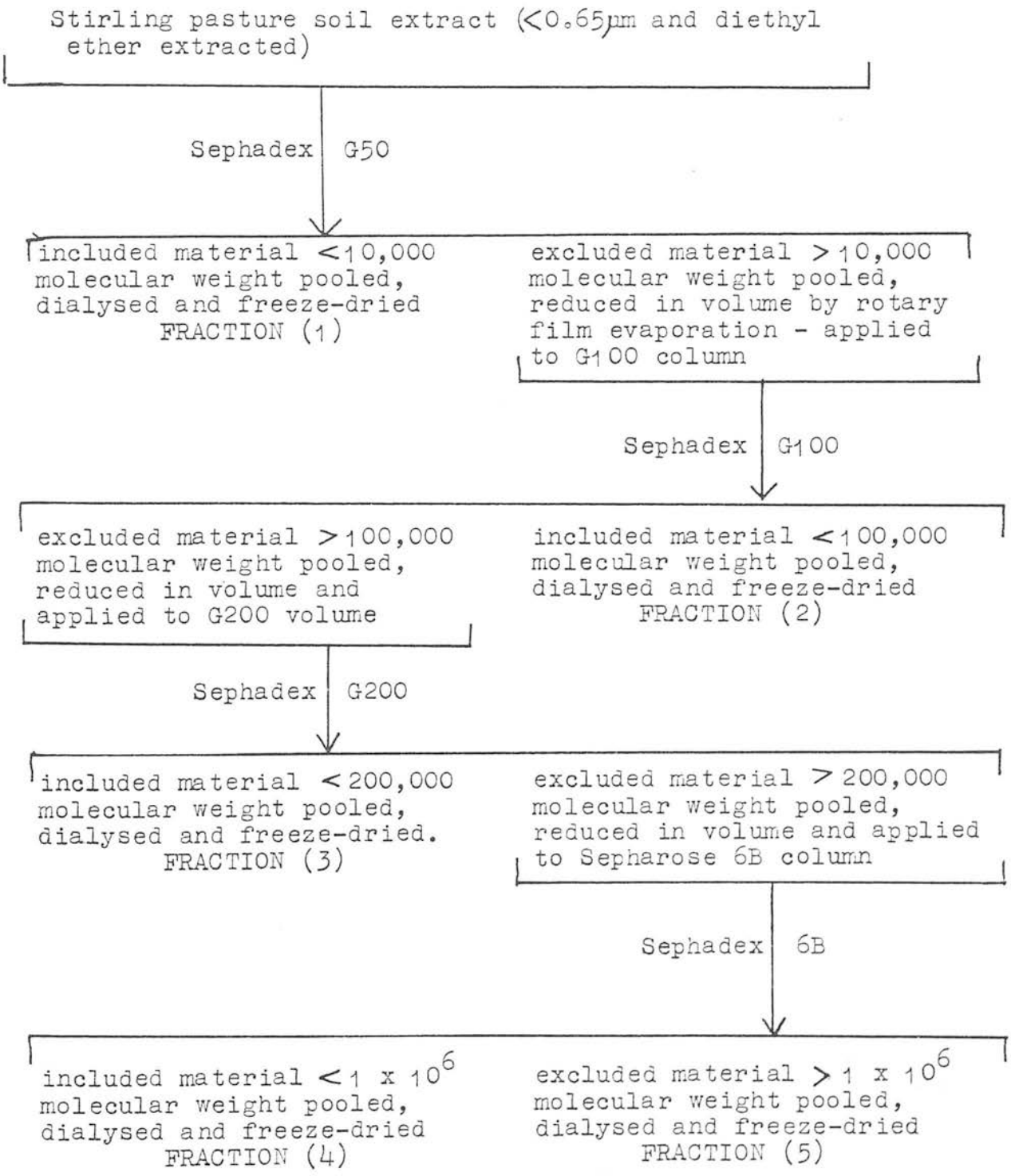


TABLE 65. The Chemical properties of organic matter fractionated on a basis of molecular weight (Stirling pasture soil)

Organic matter fraction*	Yield of organic matter (mgm-ash free basis)	% of D.M. extracted occurring in each fraction (ash free)	Ash content (%)	Total sulphur concentration (%S)	Amount of sulphur in each fraction (mgm)	Proportion of total sulphur occurring as HI-reducible S.(%)	Proportion of total sulphur occurring as carbon-bonded S.(%)
1) < 10,000 mol.wt.	141	14	1	0.37	0.53	19	81
2) 10,000-100,000 mol.wt.	93	10	5	0.54	0.53	44	56
3) 100,000-200,000 mol.wt.	486	50	4	0.14	0.68	56	44
4) 200,000 - 1×10^6 mol.wt.	170	17	6	0.15	0.26	67	33
5) > 1×10^6 mol.wt.	89	9	62	0.14	0.12	64	36

*All molecular weight ranges are nominal.

Much of the organic matter extracted (50%), is found in fraction (3) (100,000-200,000 molecular weight) with approximately equal quantities occurring in the other four fractions. All the fractions except fraction (5) possess very low ash contents probably because the organic amino-type cation of the tris buffer would be associated with the organic matter. Fraction (5) ($> 1 \times 10^6$ molecular weight) has a large ash content due to the presence of fine clay particles < 0.65 µm. As in the previous fractionation the organic matter of this fraction will be intimately associated with the mineral particles.

The two lowest molecular weight fractions contained the highest concentrations of sulphur while the remaining three fractions contained almost equal sulphur concentrations. Consequently much of the sulphur extracted is found in fractions (1) and (2). Also much sulphur is recovered from fraction (3) because this fraction contains half of the organic matter extracted from the soil. This agrees with the previous fractionation scheme which, when applied to the Stirling pasture soil, found a large percentage of the sulphur occurring in fractions containing material $<$ 200,000 molecular weight.

When the forms of sulphur in each fraction were examined a trend was seen where the percentage of the sulphur in a fraction occurring as HI-reducible sulphur increased with increase in molecular weight. Therefore, as in the previous fractionation scheme, the organic

sulphur intimately associated with the fine clay contains high percentages of HI-reducible sulphur. However this fractionation scheme demonstrates that the HI-reducible sulphur might exist as high molecular-weight compounds instead of simpler lower molecular-weight compounds attached to mineral particules. This is demonstrated because fraction (4) contains a high proportion of HI-reducible sulphur forms yet has a low ash content. This fractionation, purely on a molecular weight basis, does not show a high proportion of HI-reducible sulphur in the < 100,000 molecular-weight fractions (fractions (1) and (2)). However the previous fractionation scheme showed that a very high proportion of the sulphur, in the fulvic acid < 200,000 molecular-weight fraction, occurred as HI-reducible sulphur. This suggests that the humic/fulvic type of separation does not separate according to molecular weight alone and is of more use in grouping together similar chemical forms of organic sulphur than a strict molecular-weight based fractionation.

A further attempt to characterise the sulphur contained in the organic matter fractions was made by determining the amount of sulphate mineralised from the organic sulphur of each fraction. A known weight of each organic matter fraction was intimately mixed with purified sand in a 150 ml conical flask in addition to an inoculum of soil micro-organisms. The mixture was maintained at a suitable water content for seven days at 30°C. No net mineralisation of sulphur was observed for any organic matter fraction possibly due to the lack of metabolisable carbon.

4.6.5 The molecular weight fractionation of organic sulphur extracted from a Fenland peat

An organic soil was fractionated to allow comparison between the distribution and chemical forms of sulphur in a peat and a mineral soil. Also the preparation of a low ash content, high molecular weight ($> 10^6$ molecular weight) organic matter fraction would clarify questions raised by work performed previously with the Kilmarnock and Stirling soils (see section 4.6.3 and 4.6.4) in which this high molecular weight fraction was found to have a high ash content.

The fractionation scheme used for the Stirling soil (section 4.6.4) was also used for the peat except that the first fraction collected was that included from Sephadex G100 (< 100,000 molecular weight). Gels with exclusion limits less than 10,000 molecular weight were examined but insufficient organic material was included to allow subsequent chemical analysis. Table 66 shows some properties of the peat organic matter fractions.

The molecular weight distribution of the extracted peat organic matter differed from that of the Stirling pasture soil. The majority of the peat organic matter (59%) is less than 100,000 molecular weight whereas most of the Stirling soil organic matter occurs in the 100,000-200,000 molecular weight range. All the peat organic matter fractions possess a low ash content which would be expected of an organic soil.

TABLE 66. The chemical properties of organic matter fractionated on a basis of molecular weight (Fenland peat)

Organic matter fraction*	Yield (mgm D.M.- ash free basis)	% Ash	Sulphur concentration (%S)	Amount of sulphur (mgm S)	Proportion of the total sulphur occurring as HI-reducible S (%)	Proportion of the total sulphur occurring as carbon-bonded S (%)	Proportion of the total sulphur mineralised during a 7 day incubation (%)
1) < 100,000 mol.wt.	703	7.9	0.694	4.9	23.9	76.1	14
2) 100,000- 200,000 mol.wt.	276	5.2	0.366	1.0	55.2	44.8	22
3) 200,000- 1×10^6 mol.wt.	84	6.0	0.986	0.8	52.7	47.3	29
4) > 1×10^6 mol.wt.	130	4.4	0.787	1.0	64.0	36.0	13

*the molecular-weight ranges shown are nominal.

There appears to be no relationship between sulphur concentration and molecular weight. However much of the sulphur was recovered from the < 100,000 molecular weight fraction (64%) whereas the remaining three fractions each contributed roughly equal amounts of sulphur (approximately 10 percent). The percentage of the total sulphur in each fraction occurring as HI-reducible sulphur increased with increase in molecular weight. This trend was noted above in the Kilmarnock and Stirling soils. Therefore, because the high molecular weight fraction of the peat organic matter has a low ash content it appears likely that the HI-reducible sulphur is associated with large organic molecules as opposed to being adsorbed onto colloidal clay.

The peat organic matter fractions were incubated, as described in section 4.6.4 for the Stirling soil. All fractions showed net mineralisation of sulphur in contrast to the Stirling soil organic matter fractions which showed no net mineralisation of sulphur. The percentages of the total sulphur mineralised are high compared to whole soils incubated under similar conditions (see section 4.4) due to the finely divided nature of the organic matter of the fractions which is readily accessible to micro-organisms. It is difficult to interpret these values of mineralised sulphur since they will be dependent on factors other than the quantity of readily mineralisable sulphur present in each fraction

(for example:- amounts of readily utilisable carbon and nitrogen, rates of sulphate immobilisation).

4.6.6 The molecular weight distribution of recently incorporated sulphate-35.

Whitsome series soil, which had been incubated with sulphate-35 in the presence and absence of glucose-carbon (described above in section 3.7.1) was extracted with acetylacetone. The extraction was carried out as previously described in section 4.6.1. The extract was filtered through a 0.65 µm filter, extracted with diethyl ether, reduced in volume by rotary evaporation and made up to 100 mls with distilled water in a volumetric flask. The acetylacetone extracted 53 percent of the labelled soil sulphur from the soil receiving glucose-carbon and 58 percent from the soil not receiving glucose-carbon. Portions (20 mls) of the soil extracts were eluted down a column of Sepharose 6B using Tris buffer at pH9 as the eluant. Sepharose 6B fractionates polysaccharides in the molecular weight range of 10,000 - 1×10^6 and was therefore suitable for this fractionation. Fractions of eluant (5g) were collected for isotopic assay and optical density determination at 410 nm wavelength. Figures 26 and 27 show the elution of organic matter from the column and the amount of sulphur-35 associated with each fraction. The figures show that sulphur-35 has been incorporated into organic molecules of all molecular weights within the molecular-weight range 10,000 - 1×10^6. However the distribution of sulphur-35 shows a peak in the

Figure 26. The fractionation of Whitsome soil series (incubated without added glucose-carbon) extract on Sepharose 6B, indicating the amount of sulphur-35 associated with each fraction

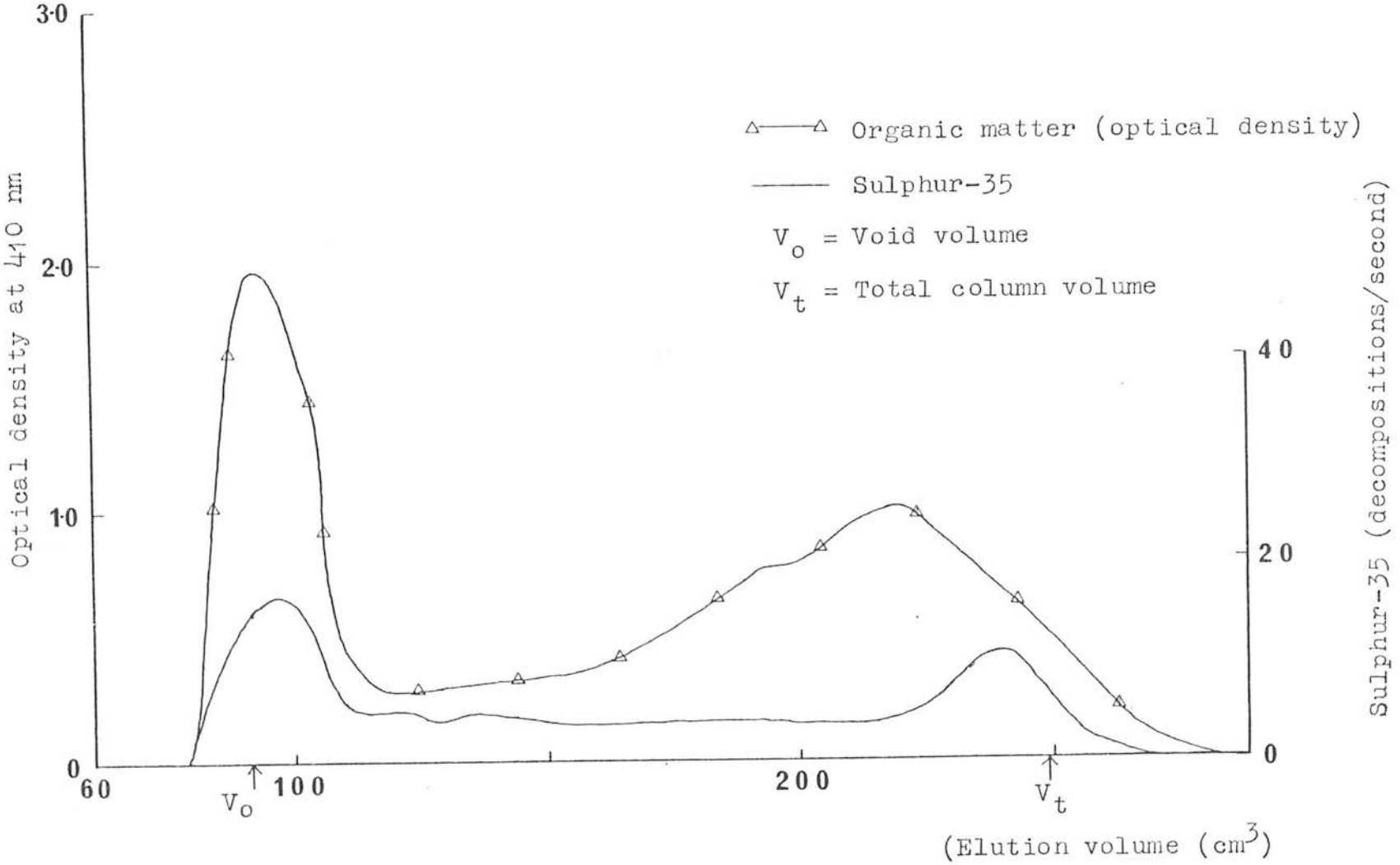
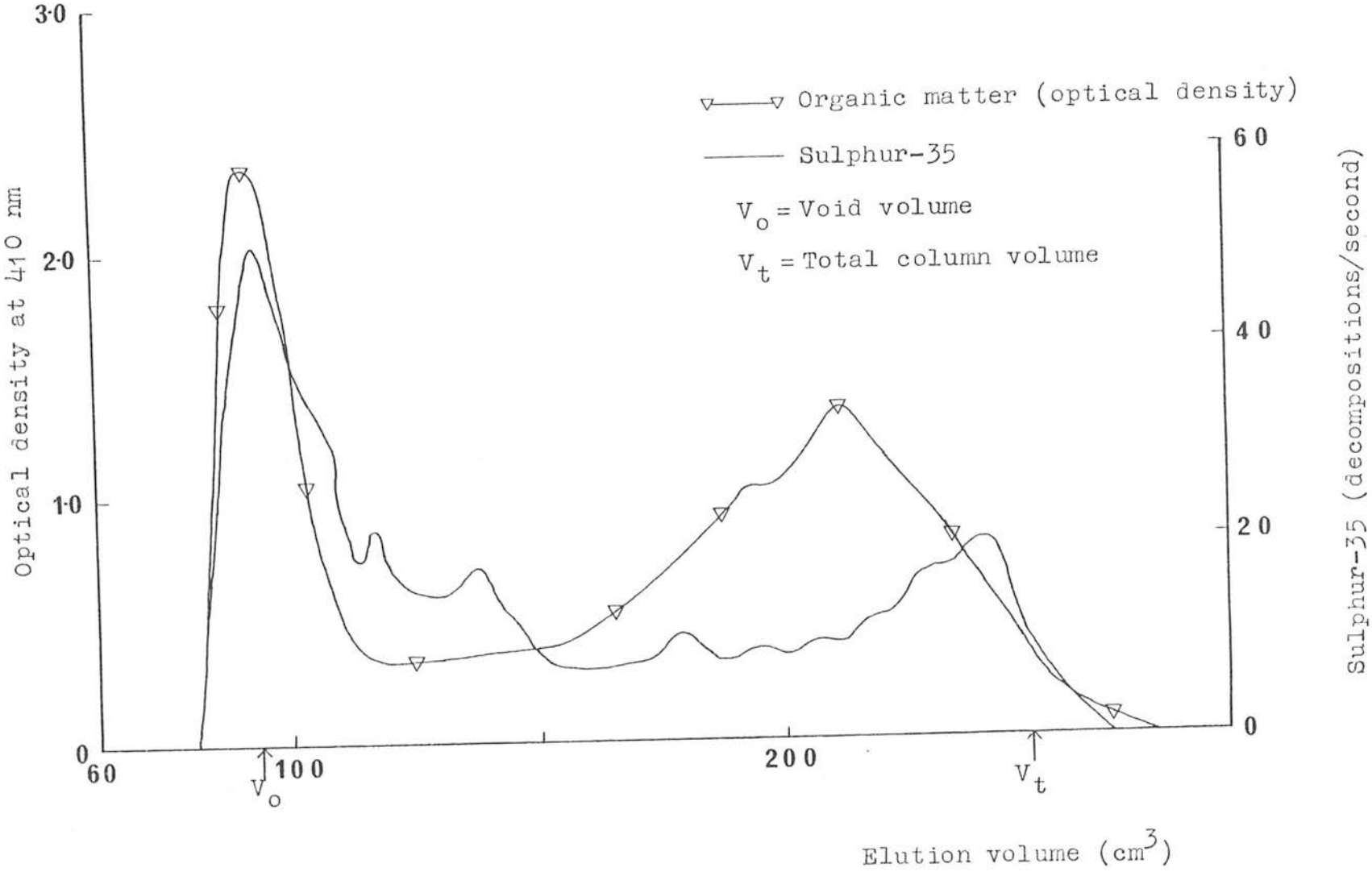


Figure 27. The fractionation of Whitsome soil series (incubated with added glucose-carbon) extract on Sepharose 6B, indicating the amount of sulphur-35 associated with each fraction.



excluded portion of the elution pattern which is especially noticeable for the soil incubated with added glucose-carbon. This peak could be due to high molecular-weight labelled compounds or possibly lower molecular-weight labelled compounds adsorbed onto fine clay particles. Only a small proportion of the mid molecular-weight range of organic molecules contained recently incorporated sulphur-35. The distributions of organic matter and sulphur-35 in the included peaks were noticeably different. The sulphur-35 peak was eluted after the organic matter peak in the case of both the soil receiving glucose-carbon and the control soil (zero carbon addition). Therefore a relatively distinct low molecular weight fraction of recently formed organic sulphur has been produced in both soils.

Since this included peak is eluted very close to the total column volume it could be due to sulphate-35 liberated by acetylacetone extraction and ultrasonic dispersion. To check that this had not occurred an attempt was made to separate sulphate-35 from low molecular weight sulphur-35 organic compounds. Initially ultrafiltration was tried using a UB2 membrane (Amicon Ltd., U.S.A.) This membrane should retain only molecules greater than 1,000 molecular weight but it was found to retain ions when used with a solution containing sulphate-35 (0.1uCi) and 10µgS/ml of carrier sulphate-32. This anomaly was probably due to electrostatic interaction between the sulphate ions and the membrane surface.

Sephadex G10 was then examined as this gel excludes molecules of molecular weight greater than 700. Sulphate-35 (0.1uCi) and 20μgS/ml as sulphate (as sodium sulphate) was applied to the column of Sephadex G 10 gel. It was found that the sulphate-32 and sulphate-35 were both eluted within the void volume and only the sodium ions appeared in the included volume as theory would predict. Again electrostatic interaction was probably responsible for the anomaly. Sephadex G 25 was then similarly tested as described above and in this case all the ionic species were eluted at the total column volume. Therefore although a technique for separating low molecular weight organic sulphur-35 compounds from sulphate-35 could not be developed the included sulphur-35 peak from the Sepharose 6B elution could be pooled and re-fractionated down a Sephadex G 25 column. About 50% of the Sepharose 6B included sulphur-35 (extracted from the soil incubated with carbon) was excluded from G 25. The remaining 50 percent could therefore be either organic sulphur-35 compounds less than 5,000 molecular weight or sulphate-35. To further characterise the G 25 included sulphur-35 it was partitioned into HI-reducible sulphur-35 and carbon bonded sulphur-35. It was found that 33 percent of the G 25 included label was carbon-bonded which indicates that by no means all of the $< 5{,}000$ molecular-weight sulphur-35 occurred as sulphate-35.

5. CONCLUSION

The field experiments conducted in this study have shown that, at present, a state of sulphur sufficiency exists in southern Scotland. This sufficiency, however, is only marginal and is dependent upon sulphur supplied by the soil. This soil sulphur was necessary to maintain sulphur sufficiency in areas of average and low atmospheric sulphur inputs. Therefore no yield response to added sulphur was observed in the field. However the addition of sulphur increased the total sulphur and sulphate content of herbage and in doing so, improved the nutritional quality. Added sulphur was found not to increase concentrations of Kjeldahl-nitrogen or have any effect on the nitrate concentrations in herbage. The sulphur budget sheets enabled the amount of sulphate, supplied by the soil from mineralisation, to be calculated. In 1977 at Dykegatehead 15kg of soil supplied sulphur/ha was removed by the plants over the growing season and in 1978, 16.7kgS/ha was removed. Similar amounts of soil supplied sulphur were removed at Blackaddermount in 1978 (18.4kgS/ha). At Boghall less reliance was placed on soil supplied sulphur although again the soil was a net sulphur contributer of 8.2kgS/ha in 1979 and 7.9kgS/ha in 1980. Soil analysis indicated that sulphur applied in the spring had been lost from the topsoil before the following spring (losses are due to plant uptake and leaching). The field experiments firmly established the

significance of soil derived sulphur indicating the importance of subsequent laboratory studies aimed at examining the mineralisation process and the labile forms of soil organic sulphur. Also whilst the field experiments gave no yield response to added sulphur, the marginal sufficiency found will need regular monitoring in the future, since sufficiency can only continue if the soil maintains these rates of sulphur supply.

The first pot experiment used soil from the field experiments at Dykegatehead and Blackaddermount. In the pot, yield responses to added sulphur were observed even where amounts of sulphur, equivalent to that supplied by the atmosphere in the field, were added. This illustrated the disparity between field and pot experiments. Yield responses of up to 36 percent were obtained from added sulphur. Since sulphur deficient herbage was obtained it allowed various sulphur status measurements to be compared. Total plant sulphur gave a poor indication of either sulphur sufficiency or sulphur deficiency. Extractable plant sulphate exhibited an improved relationship with yield response and it was found that herbage containing less than 600μgS/g D.M. was likely to respond to added sulphur. However the best indicator of sulphur status was the extractable plant sulphate expressed as a percentage of the total sulphur (where this value fell below 30 percent a response to added sulphur was likely). Added sulphur

again, did not increase concentrations of Kjeldahl-nitrogen and only where plant sulphate levels fell to 50µgS/g D.M. was there a significant increase in nitrate concentration. It was also found that added nitrogen reduced the concentration of sulphur in the herbage.

The second pot experiment showed yield responses to added sulphur for seven of the nine Scottish soils investigated. The extractable soil sulphate data correlated with yield responses at both cut 1 and cut 2 and therefore provided a good indication of soil sulphur status. Again extractable plant sulphate and the extractable plant sulphate expressed as a percentage of the total plant sulphur both proved good measures of sulphur status. The amounts of sulphur mineralised by each soil during the growth period were calculated but did not convincingly correlate with soil chemical parameters.

Initially soil sulphate mineralisation was studied using incubation techniques. All the soils examined showed an initial flush of mineralised sulphate after which an equilibrium was often attained after 50 days incubation. This flush of sulphate is probably due to the Birch effect often noticed with air dried soils. Added glucose-carbon depressed the amount of net mineralisation in all five soils examined and with the Whitsome soil net immobilisation of sulphur was observed. Increased temperature ($5^{o}C \rightarrow 30^{o}C$) increased the rate of net sulphur mineralisation in all five soils.

However it was noticed that all soils mineralised some sulphur at 5°C. No effect of added nitrogen or sulphur (or various combinations of these nutrients) on net sulphur mineralisation was observed. Therefore future experiments should examine the effects of combinations of added carbon, nitrogen and sulphur since net sulphur mineralisation probably depends on the relative availability of all three nutrients. Using a large sample of forty soils it was found that total soil sulphur and extractable soil sulphate provided the best indicator of the ability of a soil to mineralise sulphur. The soil carbon:sulphur ratio was noticeably poor for predicting amounts of mineralised sulphur.

Sulphur-35 studies were carried out to examine the fate of incorporated sulphur and determine the relative rates of sulphur mineralisation and immobilisation. Glucose-carbon, added to Whitsome soil, increased the incorporation of originally added sulphur-35 from 28 to 49 percent (during a 75 day incubation period). Isotopic assay indicated that no sulphur had been lost from the system during incubation e.g., by volatilisation. A similar amount of sulphur-35 incorporation was observed for the Stirling series soil (45 percent) but most of this incorporation had occurred during the first 10 days of incubation (35 percent). For the Whitsome soil most of the incorporated sulphur-35 was recovered from the HI-reducible organic sulphur (89 percent) although added glucose-carbon increased the proportion of label

recovered from the carbon-bonded sulphur. A similar partitioning of the sulphur-35 between the carbon-bonded and HI-reducible sulphur forms was found for the Stirling soil. For the Stirling soil the HI-reducible sulphur equilibrated with the sulphur-35 more quickly than did the carbon-bonded sulphur indicating the transient nature of the HI-reducible forms and the reserve nature of the carbon-bonded sulphur. The specific activity of the extractable soil sulphate fell with both soils and indicated mineralisation of indigenous sulphur-32. This fall in specific activity was increased by the addition of glucose-carbon and therefore caused a greater turnover of organic sulphur. The use of sulphur-35 allowed approximate calculation of the total amounts of sulphur mineralised and immobilised in each soil and showed that net mineralisation of sulphur-32 was not related to gross sulphur mineralisation. For example the Whitsome soil without added glucose-carbon gave a net mineralisation of 3µgS/g soil and gross mineralisation of 8.6µgS/g soil whereas when glucose carbon was added net and gross mineralisation were 1µgS/g soil and 10.8µgS/g soil respectively. The same phenomenon was also observed for the Stirling soil. These studies also showed that only a small fraction of the total soil sulphur was actively undergoing transformation.

The HI-reducible forms consistently showed the most transformational activity. Re-incubation of the

sulphur-35 labelled soil clearly showed that the recently incorporated sulphur was particularly susceptable to mineralisation. This observation provides a means of evaluating extracting solutions for their ability to extract labile soil sulphur. This work also demonstrated that the recently incorporated sulphur was more susceptable to breakdown to sulphate on air drying.

Soil organic sulphur was extracted and separated according to molecular weight in order to more fully understand the chemical forms and nature of soil sulphur. An extraction procedure, based on acetylacetone and ultrasonic disintegration, was found to extract reasonably large amounts of organic sulphur (58-89 percent of the total soil sulphur) in a relatively unaltered form. A gel permeation chromatographic technique was developed for use with soil sulphur extracts. The effect of long term cultivation on the molecular weight and chemical distribution of soil organic sulphur was examined. Cultivation increased the sulphur concentrations in the humic acid < 200,000 molecular weight fraction, for both soils investigated, indicating that sulphur was more resistant to mineralisation than carbon and nitrogen. The loss of sulphur from the molecular weight fractions of both soils showed no consistent pattern. The majority of the sulphur > 200,000 molecular weight was HI-reducible as was the fulvic acid sulphur < 200,000. The high proportion of HI-reducible sulphur in the > 200,000

molecular-weight fraction is due to high molecular weight sulphur containing compounds (e.g. sulphated polysaccharides) or lower molecular weight compounds intimately associated with fine clay (similar studies incorporating the use of an organic soil tend to favour the former explanation). A Stirling soil, fractionated purely on a molecular weight basis, again showed that the proportion of HI-reducible sulphur increased with increase in molecular weight. Sulphur-35 labelled organic sulphur was separated according to molecular weight and recently incorporated sulphur was found in organic molecules of all molecular weights within the range 10,000-10^6.

Future work should continue to assess the sulphur status of Southern Scotland since trends in agricultural practise and the desire for a pollution-free atmosphere will soon outdate work reported here. Incubation experiments involving the assessment of various proportions of carbon, nitrogen and sulphur on net sulphur mineralisation would complement findings reported above. Also a more detailed chemical investigation of the various soil sulphur molecular weight fractions could prove most illuminating. The sulphur-35 study also enables the development of an extraction procedure better able to assess the long term sulphur supplying power of soils.

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APPENDIX I.

The comparison of a chemical oxidation and an X-ray fluorescence method used to determine total sulphur in soils.

Eighty-four soils were randomly selected for the comparison. Details of the soils can be found in recent work by K. Chaney (1978). The chemical and X.R.F. methods employed, are described previously in sections 3.2.2.1 and 3.2.2.2 respectively.

Soil	X.R.F. (µgS/g soil)	Chemical Oxidation (µgS/g soil)
Ha	316 ± 6	340 ± 10
Hpp	441 ± 9	450 ± 20
Ka	350 ± 5	330 ± 10
Kpp	693 ± 1	700 ± 0
Ba (C.F.)	295 ± 5	290 ± 0
Ba (N.B.)	469 ± 7	515 ± 0
Bpp	832 ± 0	788 ± 2
Sa	785 ± 2	823 ± 0
Spp	634 ± 4	615 ± 5
Pa	342 ± 18	380 ± 0
Ppp	639 ± 1	625 ± 5
Ra	250 ± 10	255 ± 10
Rpp	876 ± 2	865 ± 0
Da	351 ± 11	323 ± 7
Dpp	861 ± 3	845 ± 0
Fa	374	370 ± 0
Fpp	496 ± 14	505 ± 15

Soil	X.R.F. (µgS/g soil)	Chemical Oxidation (µgS/g soil)
Sax	233 ± 3	280 ± 10
Fos	245 ± 8	275 ± 5
WINTON SOILS		
W_1	382 ± 1	363 ± 2
W_2	411 ± 0	422 ± 12
W_3	364 ± 0	392 ± 8
W_4	668 ± 1	650 ± 0
Wss	152 ± 2	150 ± 0
H_5	356 ± 1	372 ± 17
H_6	325 ± 3	330 ± 5
H_7	364 ± 3	390 ± 10
B_3	332 ± 11	355 ± 20
B_4	364 ± 0	368 ± 7
D_5	293 ± 0	325 ± 15
D_6	282 ± 1	273 ± 2
D_7	325 ± 2	340 ± 5
D_8	312 ± 7	318 ± 7
FF	678 ± 0	695 ± 15
PH	514 ± 2	515 ± 0
HFRO	451 ± 0	470 ± 5
CAP (1)	315 ± 0	290 ± 5
CAP (2)	296 ± 0	318 ± 2
ESSEX SOILS		
P_1	216 ± 1	235 ± 5
P_2	201 ± 4	205 ± 5
P_3	191 ± 1	200 ± 10

Soil	X.R.F. (µgS/g soil)	Chemical Oxidation (µgS/g soil)
P_4	260 ± 1	270 ± 10
P_5	300 ± 1	295 ± 5
P_5 (s.s.)	556 ± 2	585 ± 5
K_3W	588 ± 3	620 ± 10
K_3A	343 ± 8	320 ± 0
G_1	298 ± 0	295 ± 5
G_2	472 ± 2	445 ± 10
G_3	224 ± 0	223 ± 7
WARWICK SOILS		
B_{RA}	198 ± 2	190 ± 10
B_{RG}	413 ± 3	375 ± 5
WH_A	200 ± 3	165 ± 5
WH_G	511 ± 2	535 ± 5
WO_a	394 ± 3	350 ± 10
WO_g	952 ± 1	773 ± 2
DE_a	762 ± 2	715 ± 0
DEg	921 ± 1	923 ± 7
HUMBIE SOILS		
HL_6	340 ± 7	350 ± 0
HL_7	263 ± 4	285 ± 0
HL_8	353 ± 5	338 ± 12
HH	433 ± 5	400 ± 10
L_1	311 ± 1	295 ± 5
L_0	277 ± 4	280 ± 0
L_5	307 ± 0	280 ± 0
WH_9	281 ± 5	285 ± 5
WH_{11}	400 ± 0	400 ± 0

Soil	X.R.F. (µgS/g soil)	Chemical Oxidation (µgS/g soil)
STIRLING SOILS		
ME_1	274 ± 0	235 ± 5
WL_4	356 ± 4	343 ± 2
WL_7	453 ± 3	445 ± 5
BAL_1	440 ± 8	413 ± 7
WM_2	331 ± 1	300 ± 1
WB_3	401 ± 4	388 ± 2
DEVON SOILS		
A_1	765 ± 1	753 ± 2
A_2	577 ± 7	575 ± 10
A_4	524 ± 0	540 ± 5
A_5	624 ± 8	628 ± 7
A_6	612 ± 5	633 ± 2
B_1	520 ± 1	540 ± 5
C_1	443 ± 3	445 ± 5
C_3	1028 ± 4	1113 ± 12
C_4	525 ± 1	583 ± 2
C_5	393 ± 2	410 ± 0
D_1	484 ± 8	500 ± 10
D_3	474 ± 3	463 ± 7

An analysis of the data given above for 84 soils showed that there was no difference between the two methods, even at the 0.001 significance level. Other workers have also reported a similar good agreement between values obtained by the two different methods (Bergseth and Kristiansen, 1978).

APPENDIX II

The comparison of a chemical oxidation and an X-ray fluorescence method used to determine total sulphur in plant material.

A random sample of 44 herbage samples were analysed for total sulphur by the chemical oxidation and X.R.F. methods. The samples were taken from the field trials at Boghall, Crichton and Woodhead.

Plant sample	X.R.F. (gS/100g D.M.)	Chemical Oxidation (gS/100g D.M.)
BOGHALL CUT 1 1979		
B2	0.240	0.227
B3	0.250	0.219
B4	0.179	0.160
B8	0.201	0.168
B9	0.243	0.204
B10	0.258	0.227
B11	0.271	0.223
B12	0.274	0.217
B13	0.255	0.207
B15	0.164	0.136
B18	0.231	0.195
B19	0.247	0.202
B21	0.214	0.181
CRICHTON CUT 1		
C1	0.205	0.141
C3	0.295	0.219
C4	0.189	0.134
C8	0.165	0.118

Plant sample	X.R.F. (gS/100gD.M.)	Chemical Oxidation (gS/100gD.M.)
WOODHEAD CUT 1		
W1	0.223	0.150
W2	0.219	0.158
W3	0.261	0.195
W4	0.264	0.191
W5	0.279	0.217
W6	0.225	0.170
BOGHALL CUT 2 1979		
B1	0.318	0.255
B2	0.348	0.273
B3	0.364	0.305
B4	0.302	0.246
B5	0.312	0.243
B6	0.269	0.201
B7	0.228	0.203
B8	0.269	0.203
B9	0.334	0.257
B10	0.388	0.288
B11	0.382	0.290
B12	0.383	0.310
B13	0.273	0.210
B14	0.262	0.191
B15	0.258	0.201
B16	0.352	0.253
B17	0.291	0.216
B18	0.334	0.248
B19	0.376	0.286
B20	0.282	0.206
B22	0.357	0.267

The above table clearly showed that the two methods did not determine similar amounts of total sulphur. The chemical oxidation method gives consistently lower values than the X.R.F. method (a significant difference between methods was found at the 5% significance level). Since the X.R.F. method has compared favourably with other methods of determining total sulphur (Evans, 1970) one must assume that the chemical method underestimated total sulphur in plant material. This is probably due to loss of volatile sulphur compounds during combustion or incomplete oxidation of organic sulphur to sulphate. Plotting of the X.R.F. and chemical oxidation results gives a good straight line which can be described by:-

$$\text{XRF value (gS/100g D.M.)} = 0.0569 + 1.01361 \times \text{Chemical oxidation value (gS/100g D.M.)}$$

This equation could be used to convert chemically determined total sulphur values to amounts which would be determined by X.R.F. methods. However the confidence interval of the predicted value ($\pm$ 0.075gS/100g D.M.) is too great to accurately convert chemical values. Due to the inaccuracy of the chemical method when used to analyse plant material it was only used in Pot Experiment I (section 4.3.1) where insufficient sample was available to use the X.R.F. method.

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SULPHUR BALANCE STUDIES IN SOUTH EAST SCOTLAND

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INTRODUCTION

Sulphur deficiency in agricultural crops has never been regarded as a significant problem in Great Britain. However, a few years ago it was considered that the widespread use of S-free fertilisers and the introduction of Clean Air Acts together with increased crop yields indicated that a reappraisal of the situation was worthwhile. In southeast Scotland atmospheric SO_2 levels are considerably lower than in most other regions of Britain (Junge, 1972) and the concentration of S in precipitation is also relatively low (Stevenson, 1968). Sulphur deficiency therefore would most likely arise in such an area.

This paper describes some of the studies carried out in southeast Scotland since 1972 to examine the potential problem. The emphasis of the work is on the overall balance of S in the soil/plant system and the influence of added S on this balance. Inputs to and losses of S from the system are monitored and the effects of additions of S fertiliser on plant yield and composition and on soil

S status are assessed.

EXPERIMENTAL

Sulphur survey 1972-3

Soils were sampled from normal commercial farms throughout southeast Scotland and analysed for extractable sulphate. At many of the sites grass herbage samples were also taken and analysed for total S.

Field trials 1975

Three sites in intensive grass fields on farms in Berwickshire were selected. At each site four plots 10 m x 10 m were marked out, two were treated in April with calcium sulphate, the other two were left untreated as controls. The calcium sulphate treatment was equivalent to adding 20 ppm S to the top soil. N, P and K fertilisation and harvesting of the grass was carried out as normal by the farmers concerned. Herbage samples were taken at about monthly intervals and analysed for S and N.

Field trials 1977

In 1976 two farm sites were obtained for experimental work in Berwickshire and were sown out to Italian Ryegrass (RvP) with the intention of establishing a uniform sward before starting trials in Spring 1977. At this time (early April) eight plots (8 m x 2 m) were marked out at each site and received the following S treatments added as calcium sulphate 0 (2 plots), 2, 4, 8, 16, 32 and 64 ppm S added to the top soil; 120 kg N/ha was added to all plots as a commercial compound grass fertiliser. During the growing season five cuts of grass were taken and yields recorded. After each of the first two cuts a further 100 kg N/ha was added to all plots and 60 kg N/ha after the third cut. Samples of grass were taken for analysis at each cut and soil samples taken after the first and last cuts.

Rainfall was collected at both sites and analysed for S and at one site an automated air sampler was installed to

monitor atmospheric SO_2 concentrations.

Analytical

Soil sulphate was extracted by the method of Ensminger (1954) using a KH_2PO_4 solution containing 500 mg P/l. Sulphur in the extract was determined originally by reduction to hydrogen sulphide and titration with mercuric acetate using dithizone as indicator (Archer, 1956); more recently an automated turbidimetric method has been used (Sinclair, 1966).

Sulphate in rainwater samples, and in peroxide solutions formed by the absorption of SO_2 in the air sampler, was analysed by an automated thorin method (Persson, 1966).

Total S in herbage samples was analysed by X-ray fluorescence (Evans, 1970). Crude protein N in herbage samples was determined by the Kjeldahl method.

Nitrate-N and sulphate-S in herbage samples were extracted with cold distilled water, the nitrate was determined using an automated colorimetric method (Henriksen and Selmer-Olsen, 1970) and the sulphate using an automated turbidimetric method (Sinclair, 1966).

RESULTS AND DISCUSSION

The results of the soil S survey carried out in 1972-3 have been reported in detail (McLaren, 1975). However, several points resulting from the survey are relevant to this paper. Fig. 1 shows the range of extractable sulphate-S contents found in the survey soils. A large proportion of samples contained less than 12 mg sulphate-S/kg soil, and in Berwickshire 70% of all samples examined fell into this class.

12 mg sulphate-S/kg soil in the top soil represents about one year's uptake by a S-demanding crop such as intensively grown grass. Hence the sulphate pool in the soil must be continuously replenished to meet the annual S

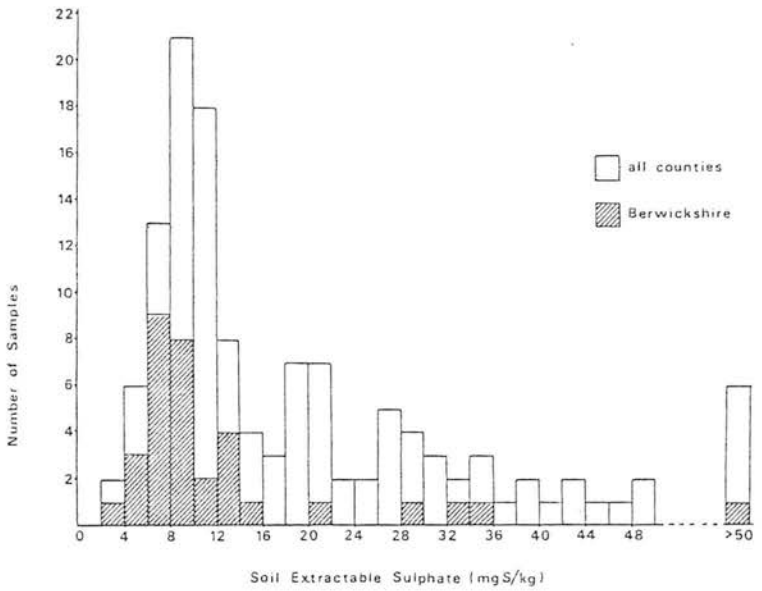

Fig. 1: Extractable sulphate in soils from S.E. Scotland

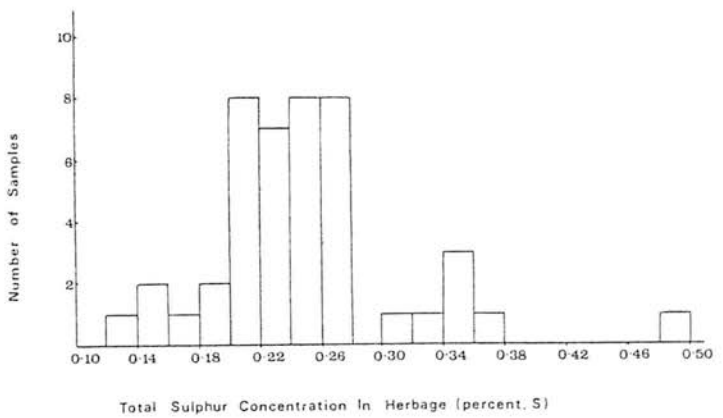

Fig. 2: Sulphur content of herbage in S.E. Scotland

requirements of crops. There would appear at present to be only two main replenishing sources, (ignoring at present the possible use of farmyard manure); (I) release of sulphate by mineralisation of soil organic matter and (2) deposition of S from the atmosphere (wet and dry deposition). The atmospheric component can, of course, also supply S direct to the plant.

The results of the S survey carried out in intensive grass crops suggests that at some sites in southeast Scotland these sources are, at best, barely coping with crop demand. Fig. 2 shows the range of total S contents found in the survey crops. Several samples contained less than 0.2% S, a level often regarded as critical for optimum S nutrition of grass and some contain less than 0.I8% S. Conserving these crops for animal food could produce diets with sub-optimum S levels.

On the basis of the survey it was decided to investigate further the S balance for intensive grass crops in southeast Scotland. Although glasshouse experiments on soils collected in the area had shown large yield responses to added S (McLaren, I975) it was considered unlikely that such responses would be obtained in the field. However, further glasshouse studies (unpublished) suggested that even if yield responses were unlikely, additions of S in the field might well improve the S status and possibly also the N status of the grass crop.

In I975 a simple trial was laid down at three sites in Berwickshire to compare the effect of adding S to the soil on the S and N content of intensively grown grass. Table I shows the total S content of the grass at the three sites. Although there were differences in response between sites the sulphur added as calcium sulphate significantly increased the S content of grass at all three sites. At site A a considerable improvement in S content was achieved especially at the time when the main silage cut was taken; S content of the control was 0.I9% and of the treated plots

0.27%. However, at site B the response was much smaller and at the time of cutting both control and treated plots were below the optimum S level, (0.II and 0.I4% respectively).

TABLE I: Sulphur content of grass at 1975 trial sites (% DM)

Sampling date	Site A		Site B		Site C	
	Control	+S	Control	+S	Control	+S
30-4	0.29	0.43	0.26	0.34	0.34	0.39
I4-5	0.25	0.35	0.22	0.28	0.32	0.35
28-5	0.I9	0.27	0.I5	0.I8	0.22	0.28
II-6	silage	cut	0.I3	0.I5	0.I9	0.2I
25-6	0.27	0.34	0.II	0.I4	silage	cut
9-7	0.I8	0.20	hay	cut	0.2I	0.26
23-7	0.20	0.24	---	---	0.22	0.26

The N content of the grass (crude protein-N) increased significantly at sites A and B as a result of the S treatment (Fig. 3). At site B at the time of cutting the increase in crude protein-N was as much as 2I% and at site A at the silage cut I3%.

Such increases in S and N content represented a considerable potential improvement in the nutritional quality of the grass, further field studies under more controlled conditions were planned. Details of the experimental design for I976-77 are given in the 'experimental' section. The main aims of this experiment were, firstly, to study the effect of increasing amounts of S on the growth and quality of intensively grown grass, and, secondly by monitoring S inputs and losses attempt to produce a S budget for the soil/crop system.

The results for total yield and S and N uptake over the whole season at site A are shown in Table 2. The S treatments had no effect on yield but significantly

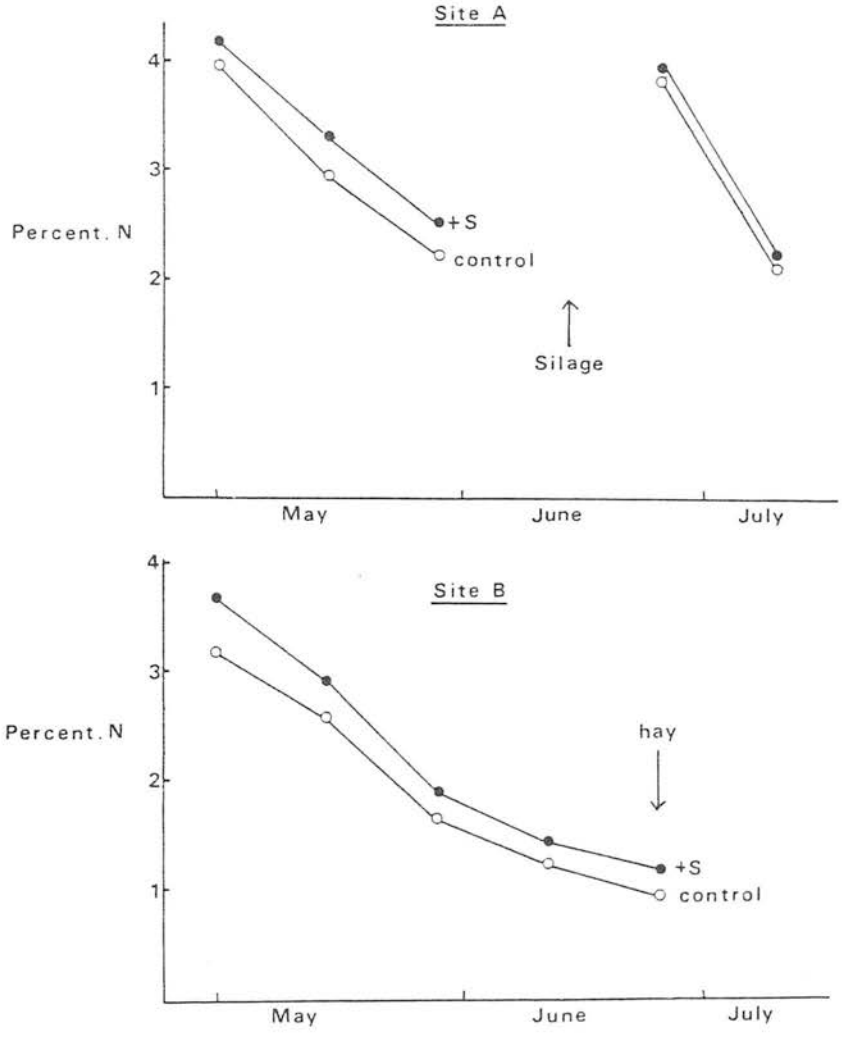

Fig. 3: Crude protein-N of grass at field sites, 1975

TABLE 2: Dry matter yield, sulphur uptake and crude protein nitrogen uptake of grass at site A (1977)

Calcium sulphate treatment (mg S/kg top soil)	DM yield t/ha	Total S uptake kg S/ha	Sulphate-S kg S/ha	Organic-S kg S/ha	Crude protein N kg N/ha
0	16.7	33.25	13.20	20.05	296.5
2	16.3	38.23	14.68	23.55	314.5
4	16.1	35.83	14.52	21.31	290.1
8	16.3	39.43	16.60	22.83	300.4
16	15.3	40.67	18.40	22.27	284.5
32	16.2	45.35	22.75	22.60	271.3
64	16.1	52.74	30.59	22.15	334.2

increased the amount of S taken up by the crop. At the first cut, which accounts for nearly 50% of the total yield, the highest S treatments considerably improved the S concentration in the grass (Fig. 4). The control and the two lowest S treatments were well within the critical range for both plant and animal nutrition (0.15-0.16% S), whereas the S content of the highest treatment was increased to 0.27% S.

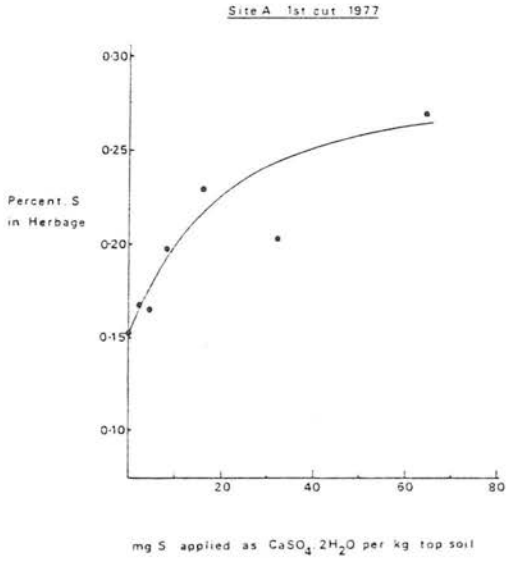

Fig. 4: Effect of sulphur treatment on sulphur content of grass

Sulphate and nitrate levels in the herbage showed that sulphate-S increased with S treatment (Table 2). Indeed much of the increase in total plant S could be accounted for by this fraction. However, no consistent relationship was observed between nitrate and sulphate levels in the plants. There was no evidence for nitrate accumulation in the low S treatments and it did not appear that lack of sulphur was limiting nitrate utilisation. Even so, there

there was a suggestion of an increase in crude protein-N at the highest rate of S addition (Table 2). It was not possible to record yields at site B but trends in S and N concentrations in the grass were similar to those found in site A.

Observations on the total S budget at site A proved interesting. It was discovered that the compound fertiliser used for the NPK treatment did, in fact, contain a small percentage (less than I%) of S which, at the high rate of fertiliser application contributed a significant amount of S to the overall budget. This might explain the poor yield response to the S treatments found in this experiment. However, even with this unintended addition of S, the grass on the control plots appeared to obtain 35% of its S from non-fertiliser or atmospheric sources (Table 3). Since extractable sulphate levels in the soil had not decreased significantly during the growing season this S must have been provided by the mineralisation of soil organic matter. The total S content of this soil is approximately 1280 kg S/ha of top soil, thus mineralisation of II.5 kg S/ha represents less than 1% of the total, which appears quite feasible.

TABLE 3: Sulphur budget for grass crop at site A (April-September 1977) (kg S/ha)

Crop removal	S added in gypsum and fertiliser	S added in rain	S in dry deposition	Balance
33.3	10	5.5	6.3*	11.5 deficit
38.2	15	5.5	6.3	11.4 deficit
35.8	20	5.5	6.3	4.0 deficit
39.4	30	5.5	6.3	2.4 excess
40.7	50	5.5	6.3	21.1 excess
45.3	90	5.5	6.3	56.5 excess
52.7	170	5.5	6.3	129.1 excess

*Calculated on basis of mean SO_2 level of 10 $\mu g/m^3$ deposition velocity 0.8 cm/sec

With the three highest S treatments the S inputs were far in excess of the S removed in the crop. Analysis of soils from the plots sampled at the end of the growing season showed considerable amounts of sulphate still present (Fig. 5). However, Fig. 5 also shows that most of this sulphate disappeared from the soil during the winter, probably through leaching, since little was likely to be incorporated into the soil organic matter during the winter. Indeed results from another experiment in which large amounts of S were applied to the soil every year for 20 years showed no significant accumulation of S in the soil from S additions alone (McLaren, 1976). Increased levels were found only where organic matter values had been raised as a result of N additions. Under these conditions immobilisation of S and improvement of the soil S status could occur.

It seems unlikely that much S deposited on the soil during winter by dry and wet deposition or remaining from fertiliser treatment will remain in the soil for crops the following season. Hence it seems sensible to confine S budget calculations to S inputs that arrive during the growing season.

CONCLUSIONS

These studies suggest that supplies of S for intensively grown grass in some areas of southeast Scotland are at best marginal. Although yield responses to added S have not been obtained in the field, some improvements in the nutritional quality of the grass crop have been observed. The overall agricultural significance of such improvements requires further study.

Any attempt to assess the present or future S nutrition of crops requires a detailed knowledge of the overall S budget in any particular situation. The contribution of atmospheric sources of S to crop nutrition can be fairly readily measured, however, the possible contribution of the soil organic matter fraction requires further study.

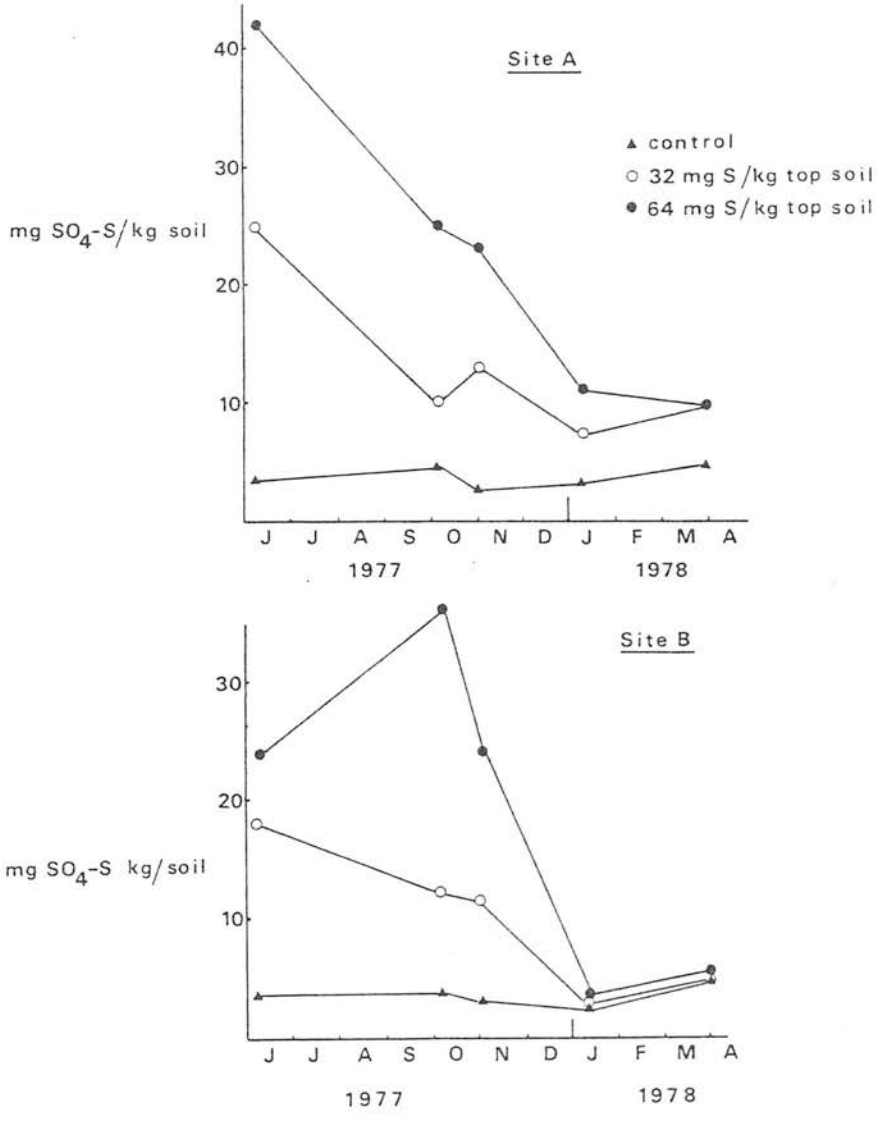

Fig. 5: Soil sulphate-S levels in experimental plots, 1977-78

Extensive mineralisation of S is known to take place in arable soils in the long-term (McLaren and Swift, 1977) but the importance of short-term mineralisation and immobilisation of S is far from clear. The results of the S balance described above suggest that mineralisation of organic S may play an important part in helping to maintain the S nutrition of a crop where atmospheric inputs are low. The important question perhaps is whether organic supplies of S are being continuously run down or whether some significant replenishment of organic supplies takes place side by side with mineralisation.

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DISCUSSION

Dr. L.H.P. Jones, England

I have two questions. First, do you have figures for C, N and S in the organic fraction of the soils used in your field experiments ? Second, what are the cropping histories of the soils ?

Dr. R.G. McLaren, Scotland

The C, N and S contents of the soils from the sites in the 1977 field experiments are :

Site A	2.27% C	0.21% N	512 ppm S
Site B	1.98% C	0.19% N	408 ppm S

Both sites are on predominantly arable farms where fields are periodically put down to intensive grass for 2-3 year breaks. Both sites were relatively near to the steadings and have probably received a considerable amount of farmyard manure over the years. The application of manure plus the fact that organic S mineralises more slowly than N and C (Swift, 1977, McLaren and Swift, 1977) could well account for the relatively low C:S and N:S ratios in these soils.

Swift, R.S., 1977. Mineralisation of N and S from soil organic matter: a comparison of pasture and arable soils. Int. Symp. on Soil Organic Matter Studies, Brunswick, Germany. Vol. I., 275-281, IAEA, Vienna.

Dr. [illegible], Northern Ireland

I've noticed that various nutrients in the soil become less available in the coldest months of the year. They recover in the spring. Is it likely that the phosphate-extractable sulphate in Fig. 5 would have shown an increase and a residual effect if you had continued measurements till April and May ?

Dr. R.G. McLaren

Extractable sulphate was in fact monitored in these soils into April and May and showed no increase in levels over those observed in the winter months.

We consider that the sulphate has been leached out of the soil and have results from a long term experiment to support this view. In this experiment large amounts of sulphate were added to a soil each year for more than 20 years and yet there was no build up of sulphate, or organic S in the soil. In addition, very little of the added sulphate was removed by the crop (grass) each year (McLaren, 1976).

McLaren, R.G., 1976. Effect of fertilizers on the sulphur content of herbage. Journal of the British Grassland Society, 31, 99-105.